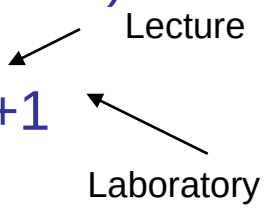
CDL Advances Chemical Studies (ACS)

Metabolic Biochemistry 7 CFU = 6+1



Prof.ssa Alessandra Olianas
Prof.ssa Francesca Pintus

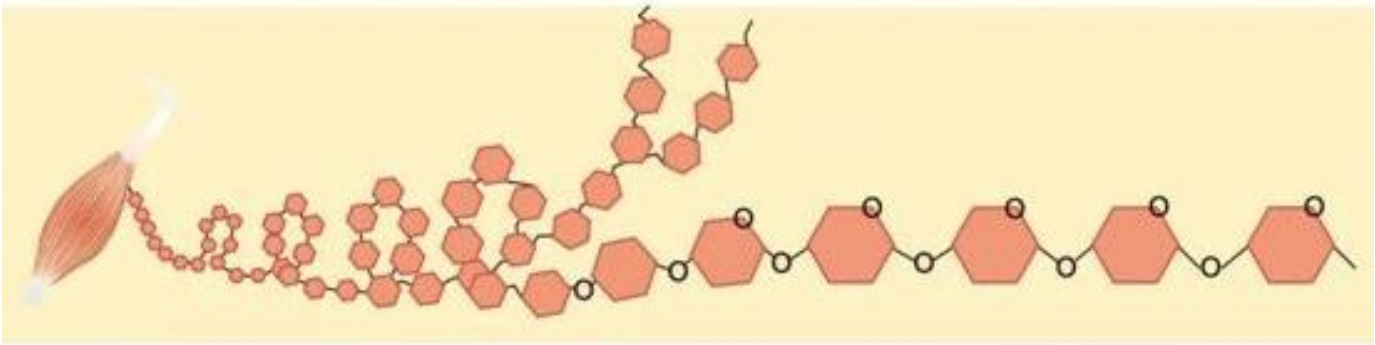
Prof.ssa Alessandra Olianas

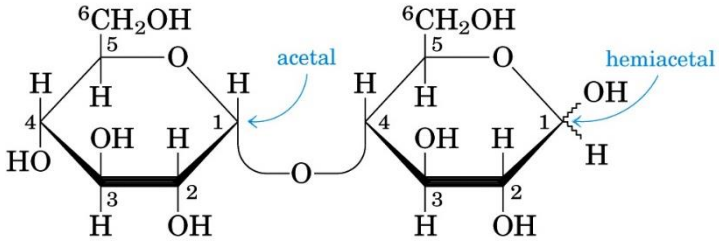
Department of Life and Enviromental Science
Biomedical Section (Biochemistry Laboratory)

Dip. Scienze della Vita e dell'Ambiente
Sezione Biomedica (laboratorio di Biochimica)
Tel. 0706754507
Students reception: by appointment

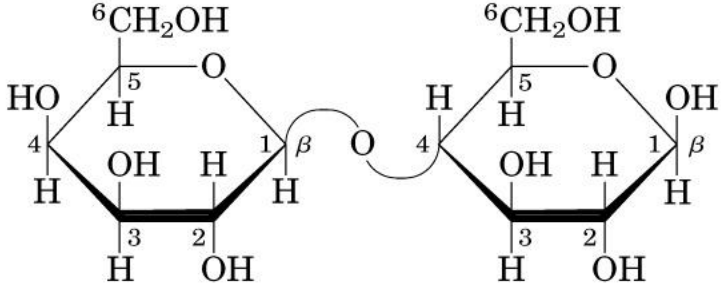
olianas@unica.it

Metabolism of Carbohydrates





Maltose
α-D-glucopyranosyl-(1→4)-D-glucopyranose



Lactose (β form)
β-D-galactopyranosyl-(1→4)-β-D-glucopyranose
Gal(β1→4)Glc

Digestion and Absorption of carbohydrates

Major carbohydrates consumed daily with the diet are mono- di- and polysaccharides

Fructose	Fruit, honey
Glucose	Fruit, honey, grape
Amylopectin	Potatoes, rice, maize, bread
Amylose	Potatoes, rice, maize, bread
Sucrose	Table sugar, sweets
Trealose	Young mushroom
Lactose	milk and dairy products
Raffinose	Legume seeds

Digestion and Absorption of carbohydrates

Monoccharides such as glucose and fructose are absorbed directly.

Di-saccharides require enzyme of the small intestinal cells

Poly-saccharides require enzyme (pancreatic amylase) or other enzyme on intestinal surface.

Digestion of carbohydrates

Carbohydrates begin digestion in the mouth

Starch and glycogen are digested by α *salivary amylase*

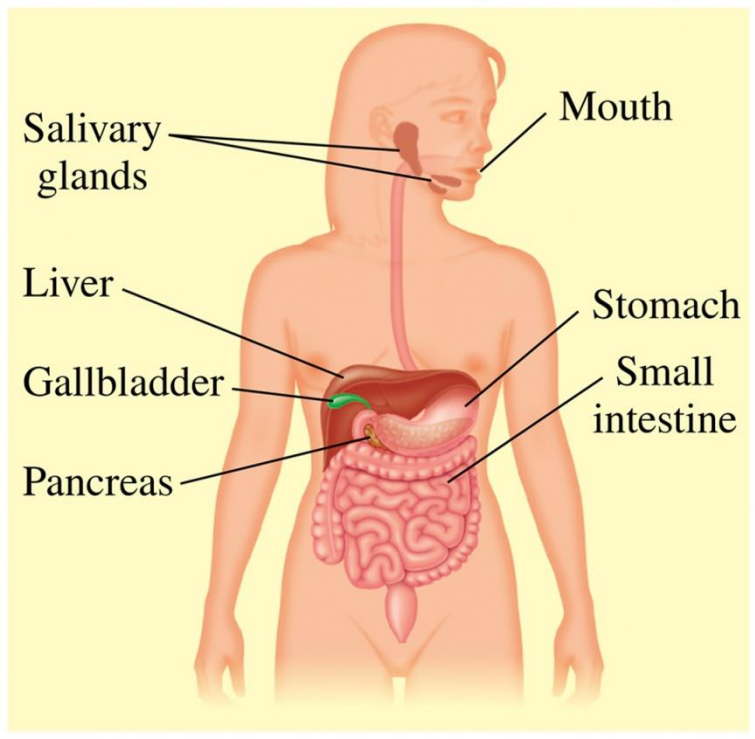
breaks down polysaccharides into smaller polysaccharides (dextrins), maltose, and some glucose.

α(1→4) glycosidic

2) α-amylase is inactivated by the low pH in the stomach

3) Small intestine:
pancreatic α-amilase continues the breakdown process

Other α(1→4) glycosidic bonds



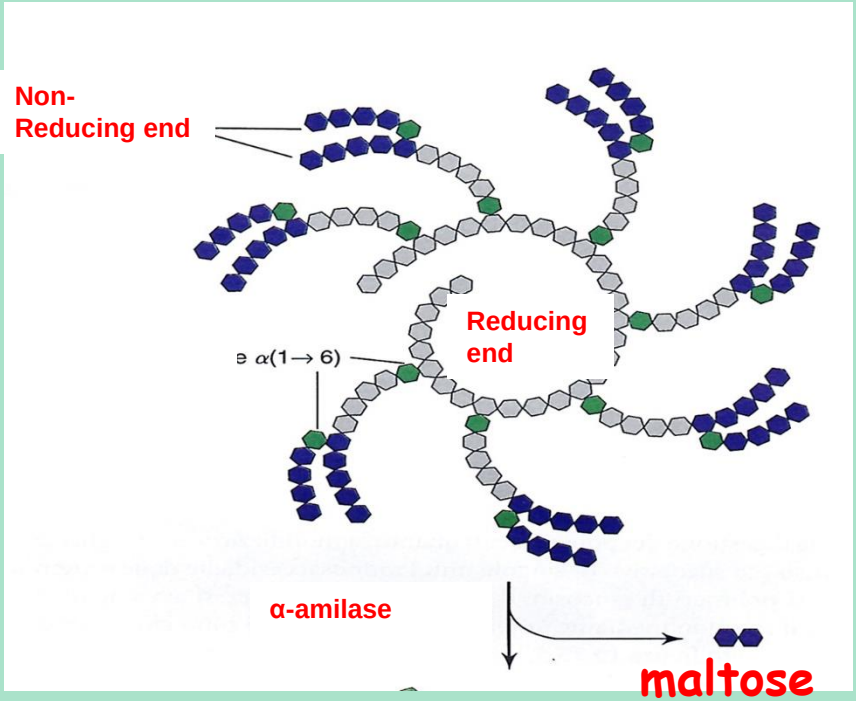
Digestion of carbohydrates

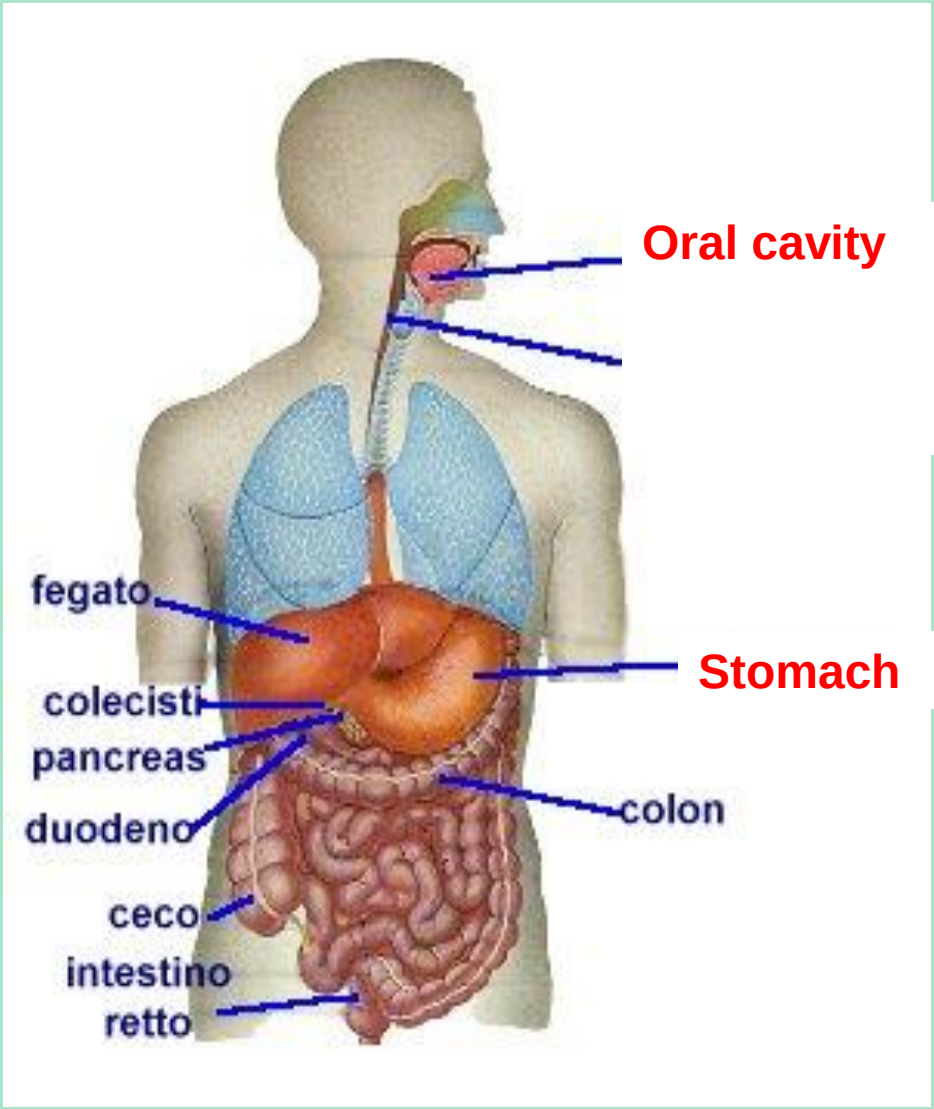
Pancreatic α-amilase

The products are mainly:

Maltose
Maltotriose
dextrins

trisaccharide

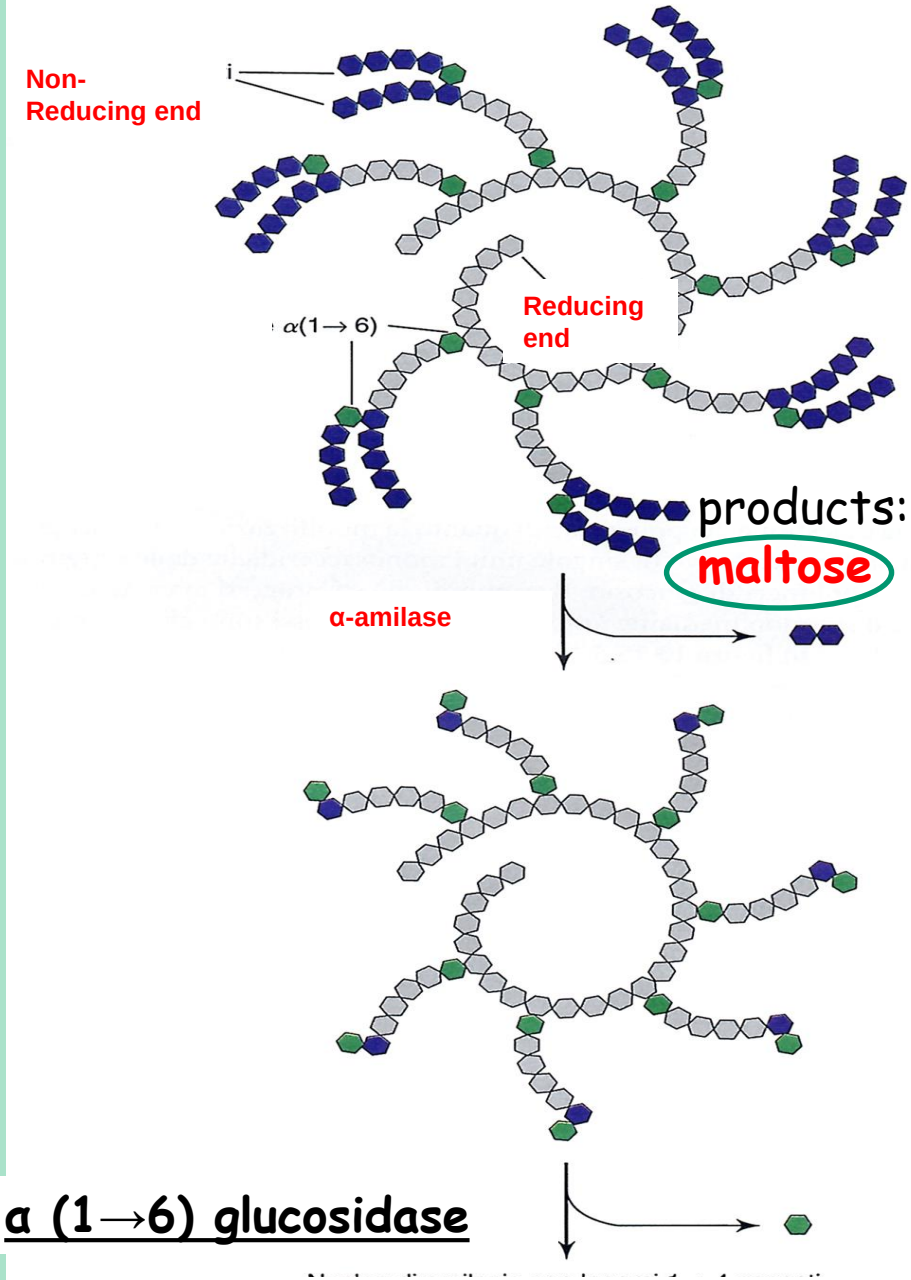




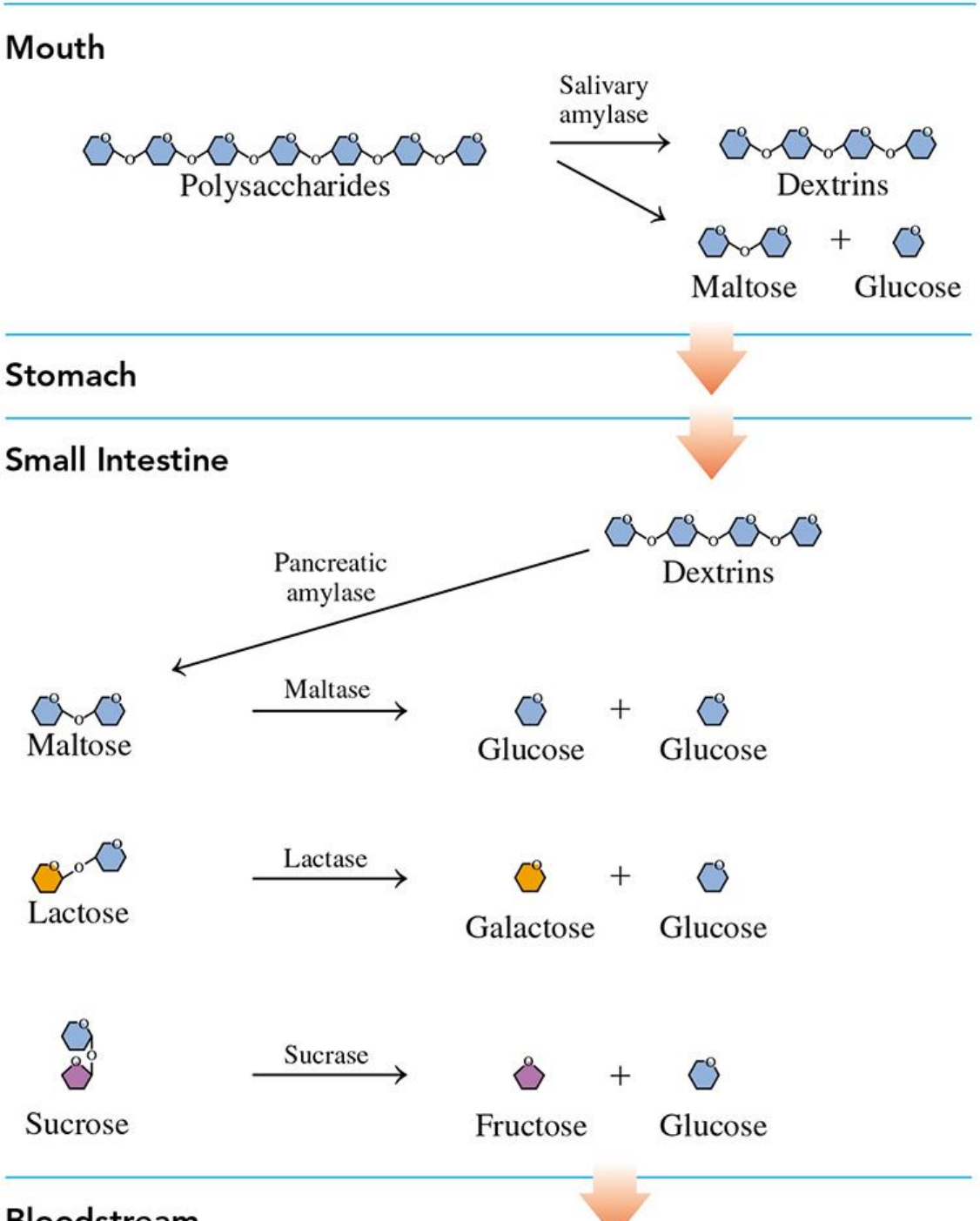
α-Dextrins: about 8 glucose units contain at least one α(1→6) glycosidic bond

Digestion are completed by enzyme product from Brush border intestinal α- glucosidase

	Enzyme	
maltose	maltase	→ Glucose(α1->4)Glucose
saccharose	saccharase	→ glucose (α1->β2)fructose
lactose	lactase	→ galactose (β1->4) + glucose
dextrins	α (1→6) glucosidase o dextrinase	



α (1→6) glucosidase


Mouth
Salivary amylase
Polysaccharides
Dextrins
Maltose
+
Glucose
Stomach
Small Intestine
Dextrins
Pancreatic amylase
Maltase
Maltose
Glucose
+
Glucose
Lactase
Lactose
Galactose
+
Glucose
Sucrase
Sucrose
Fructose
+
Glucose

Glucose Transporters

GLUTs 14 types

Sodium and ATP-independent

SGLTs

Sodium dependent

Require ATP

GLUT1
Blood,
Heart

GLUT2
Small intestine,
Liver,
Pancreas

GLUT3
Brain, Neuron

GLUT4
Skeletal muscle,
Adipose tissue,
Heart

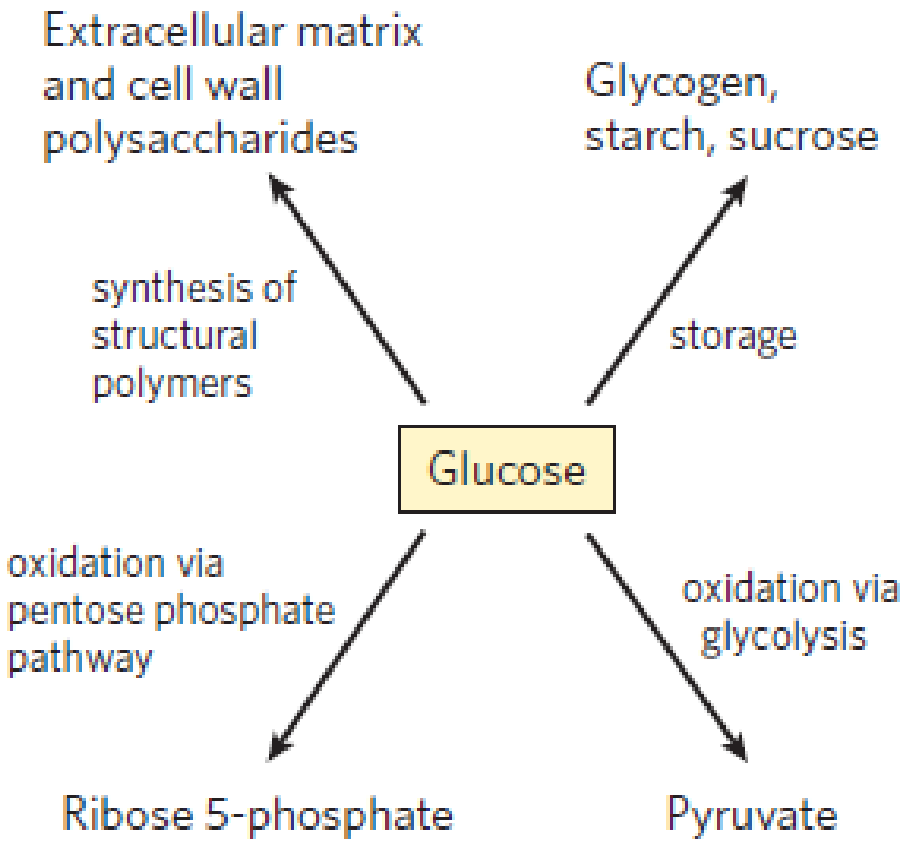
GLUT5
Enterocyte
(Fructose transporter

SGLT1
Enterocyte

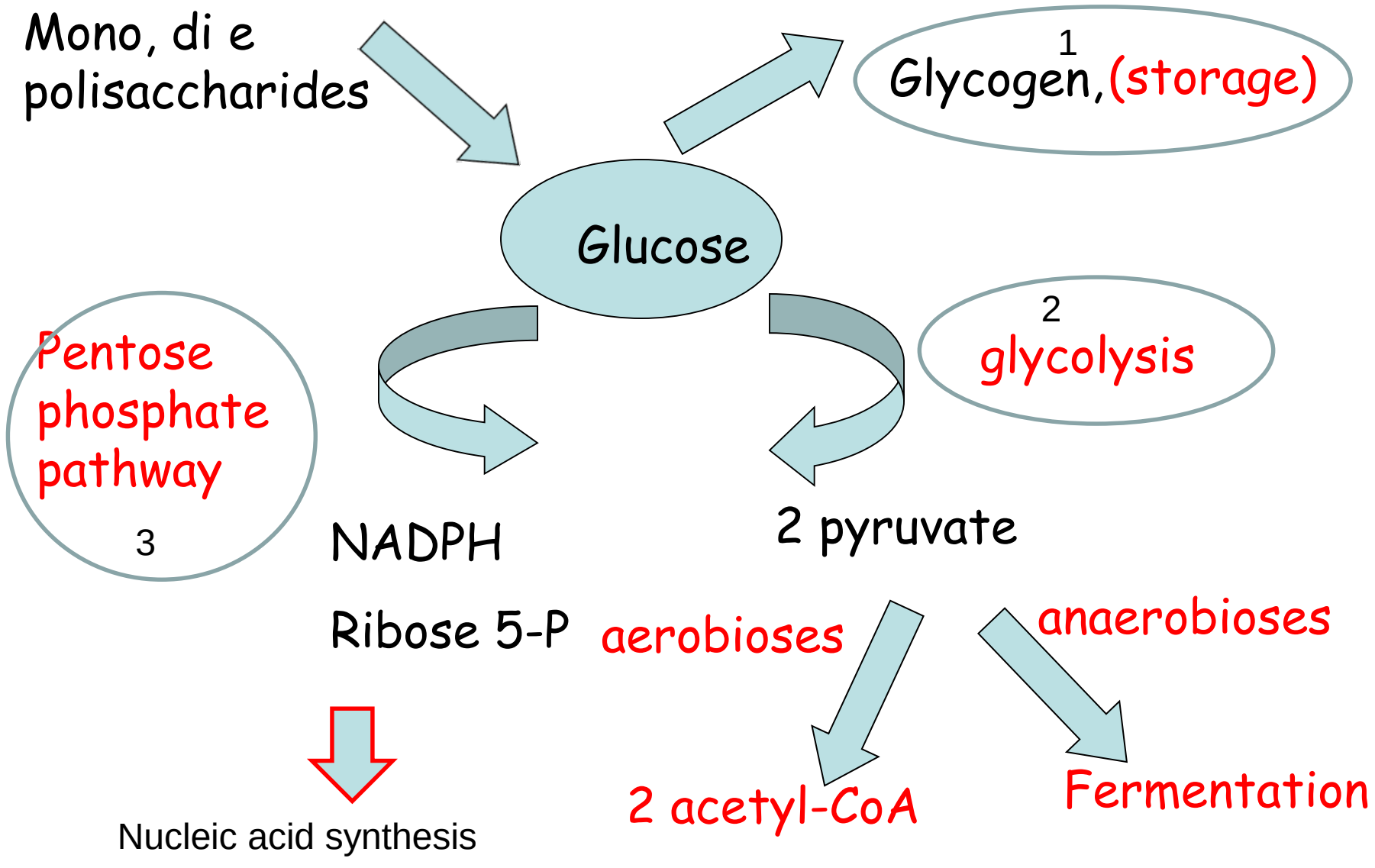
SGLT2
Kidney

Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms.

Four pathways are the most significant



Glucose introduced through the diet has three fates:



Glycolysis

Glycolysis is a metabolic pathway that occur in cytosol And converts glucose in two pyruvate molecules generating metabolic energy to form ATP.

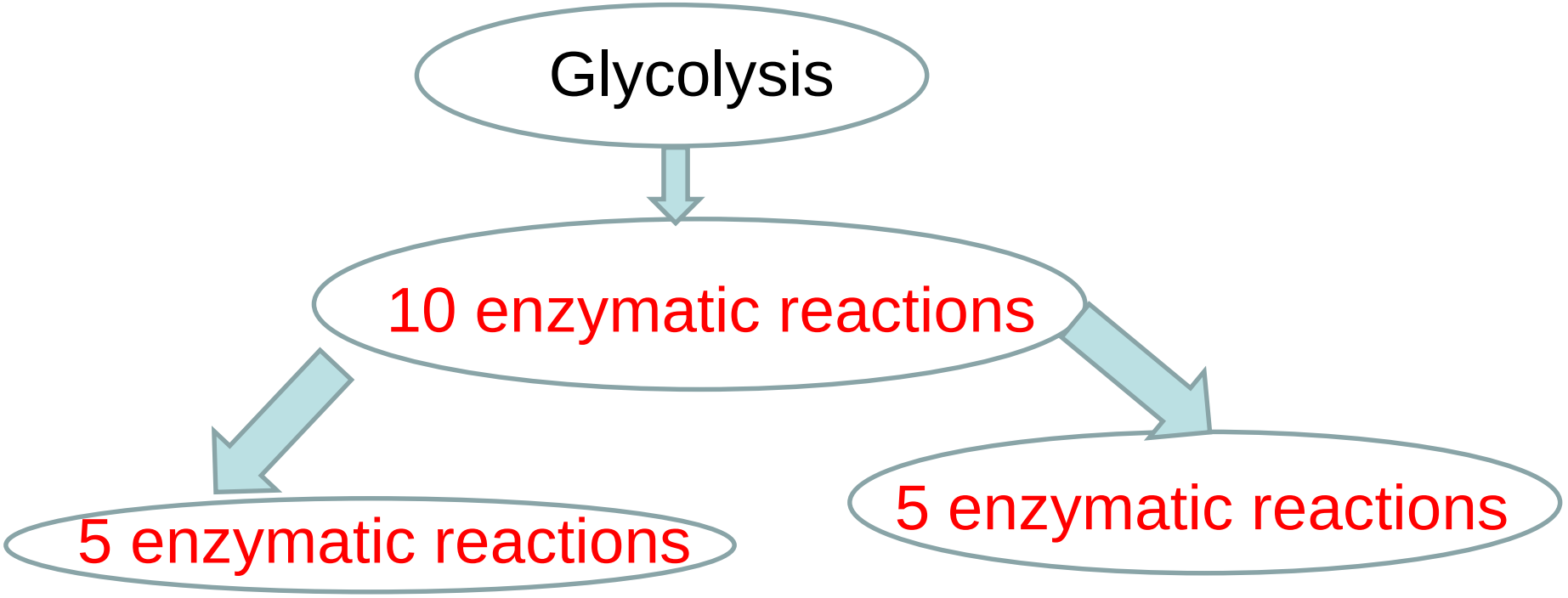
Glycolysis precedes the Krebs cycle and the electrons transport chain, (in the aerobic organisms) with glucose is completely oxidated to H_2O and CO_2.

$$C_6H_{12}O_6 + 6O_2 \gg\gg 6CO_2 + 6H_2O$$

$\Delta G'^\circ$ = -2840 kJ/mol (32 ATP molecules)

GLYCOLYSIS Greek words: Glykys = sweet

Lysis = breakdown/ splitting

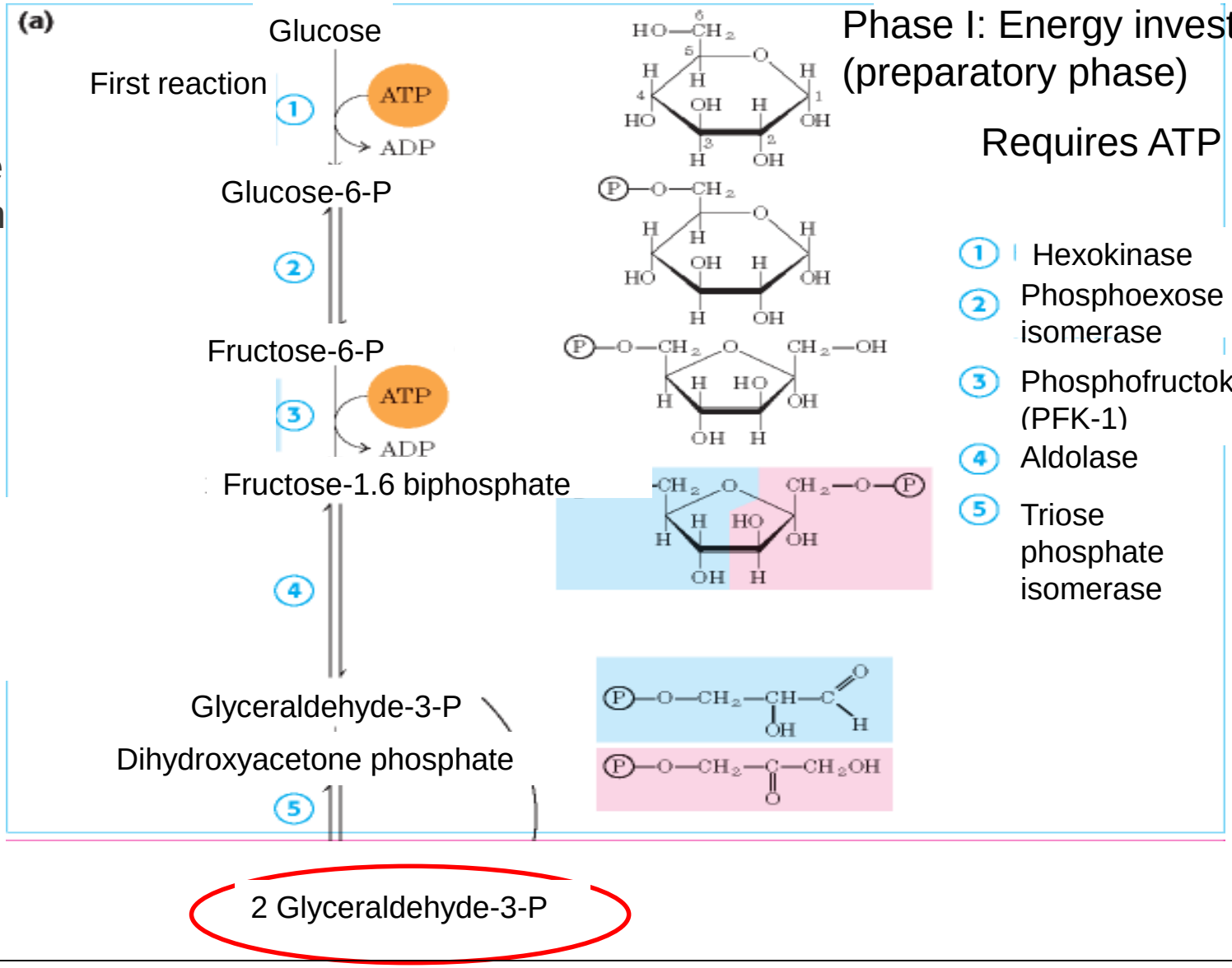


Phase I: Energy investment
(preparatory phase)

Phase II: Energy recovery
(Pay off)

GLYCOLYSIS is the sequence of 10 enzyme-catalyzed reactions that converts glucose into pyruvate with simultaneous production of ATP.

First phase requires an investment of 2 ATP molecules

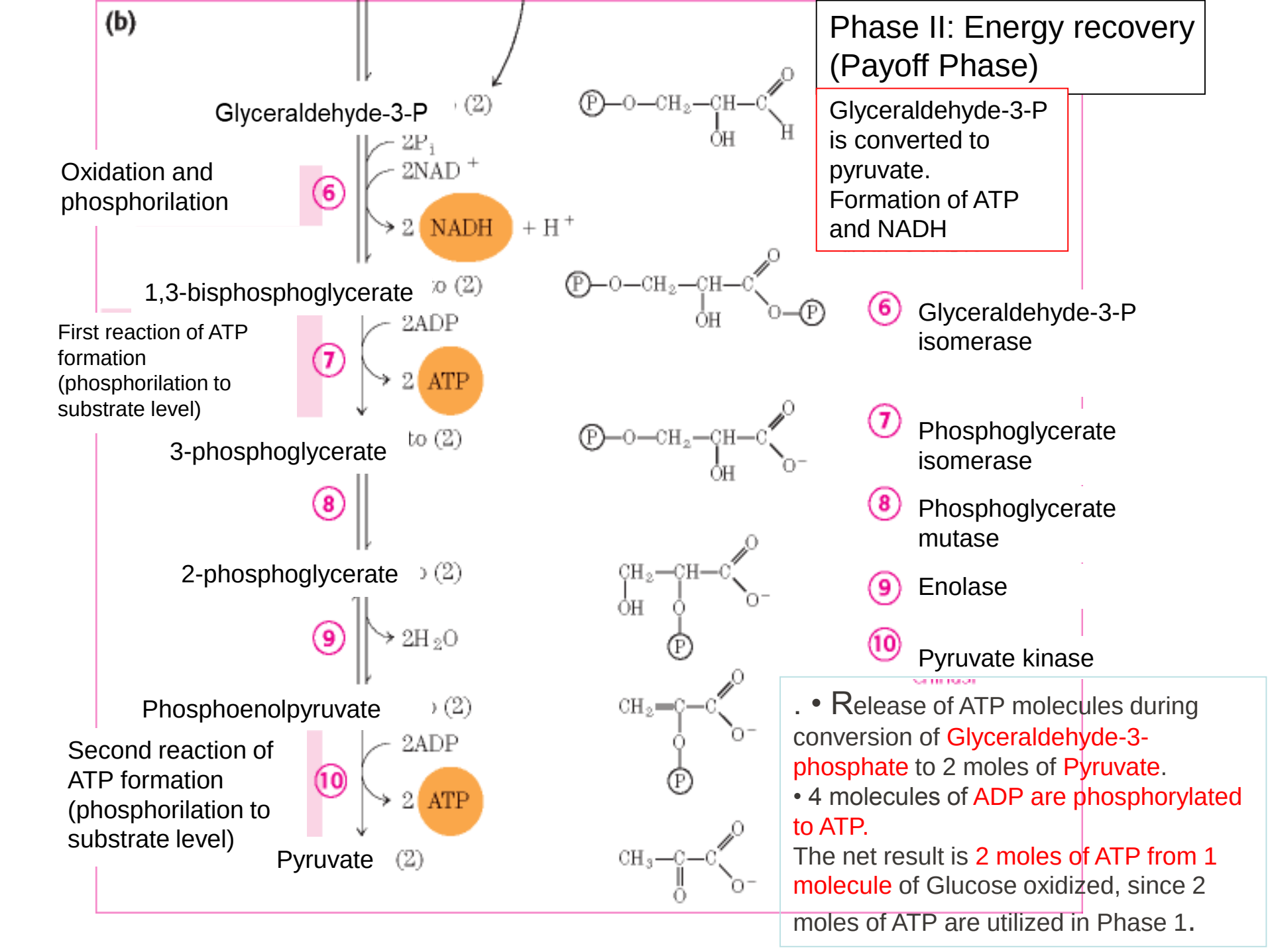


Phase I: Energy investment (preparatory phase)

Requires ATP

1. Hexokinase
2. Phosphoexose isomerase
3. Phosphofructokinase 1 (PFK-1)
4. Aldolase
5. Triose phosphate isomerase

- It consists of the 1st 5 steps in which the glucose is enzymatically phosphorylated by ATP to yield Glucose 6-phpsphate and Fructose-1,6-biphosphate.
- Fructose-1,6-biphosphate is then split in half to yield 2 molecules of 3-carbon containing Glyceraldehyde-3-phosphate/ dihyroxyacetone phosphate.



Phase II: Energy recovery (Payoff Phase)

Glyceraldehyde-3-P is converted to pyruvate.
Formation of ATP and NADH

6 Glyceraldehyde-3-P isomerase

7 Phosphoglycerate isomerase

8 Phosphoglycerate mutase

9 Enolase

10 Pyruvate kinase

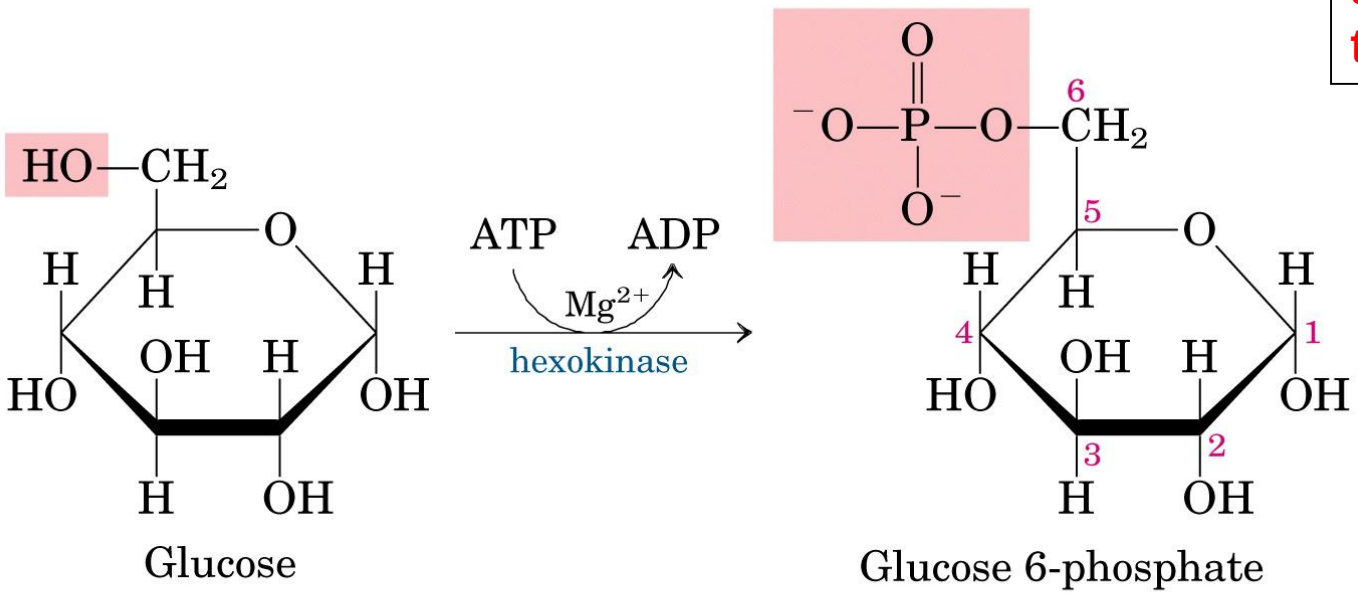
. • Release of ATP molecules during conversion of Glyceraldehyde-3-phosphate to 2 moles of Pyruvate.
• 4 molecules of ADP are phosphorylated to ATP.
The net result is 2 moles of ATP from 1 molecule of Glucose oxidized, since 2 moles of ATP are utilized in Phase 1.

Reaction 1: First ATP Utilization

Transfer of a phosphoryl group from ATP to glucose

Enzym: Hexokinase (HK)

Kinases are a subclass of transferases



Kinases requires Mg++ for its activity

Irreversible reaction

$$\Delta G'^{\circ} = -16.7 \text{ kJ/mol}$$

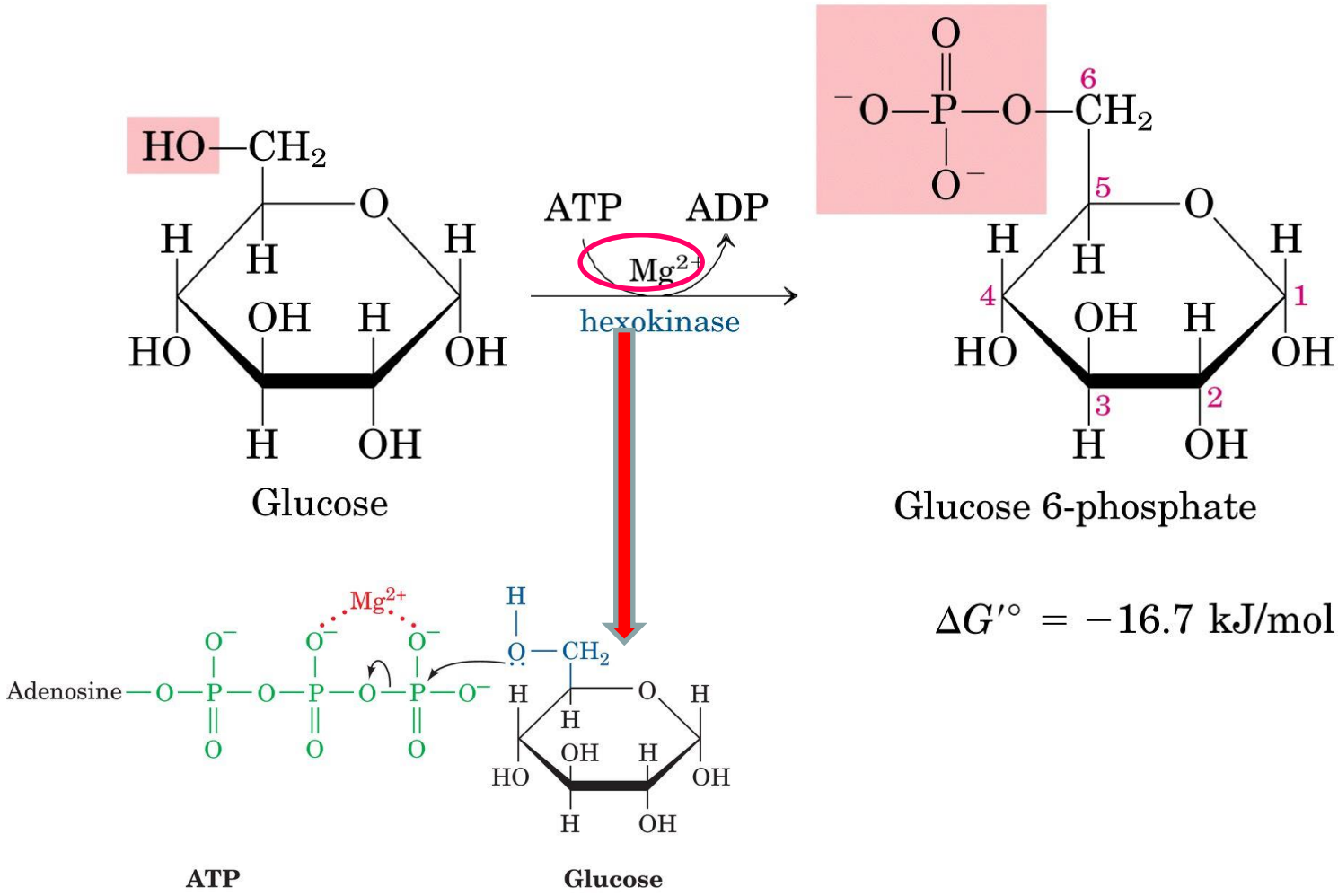
Glucose is phosphorylated by ATP to form glucose 6-phosphate.

- This is an irreversible reaction (catalyzed by hexokinase).

Glucose (enters the cell by hormonal stimulation) and i is immediately phosphorilated at 6 position.

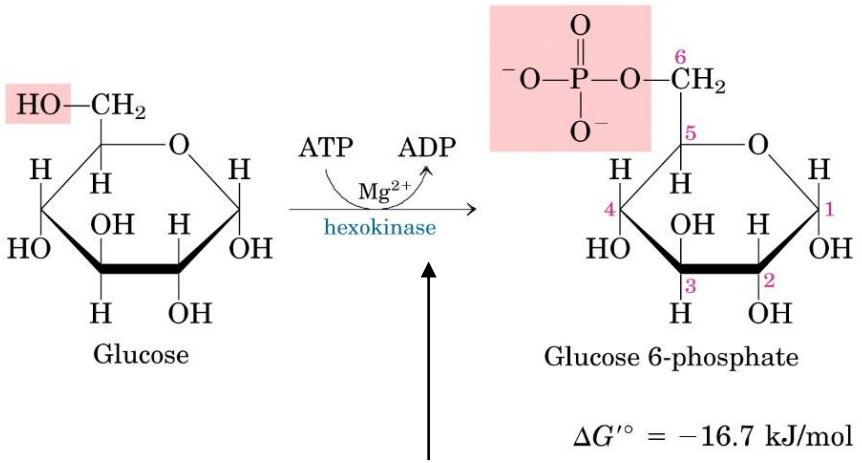
The reaction is catalyzed by glucokinase in liver and **hexokinase in muscle and brain.**

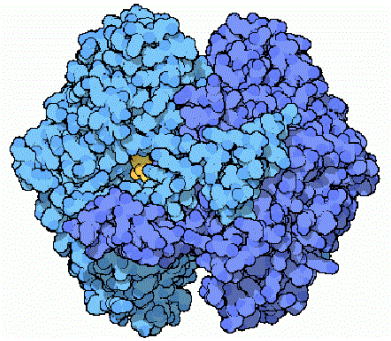
Kinases transfer a phosphate group from ATP to substrates.



Hexokinase belongs to the class of Transferases

The suffix hexo is relative to the substrate, a sugar with six carbon atoms



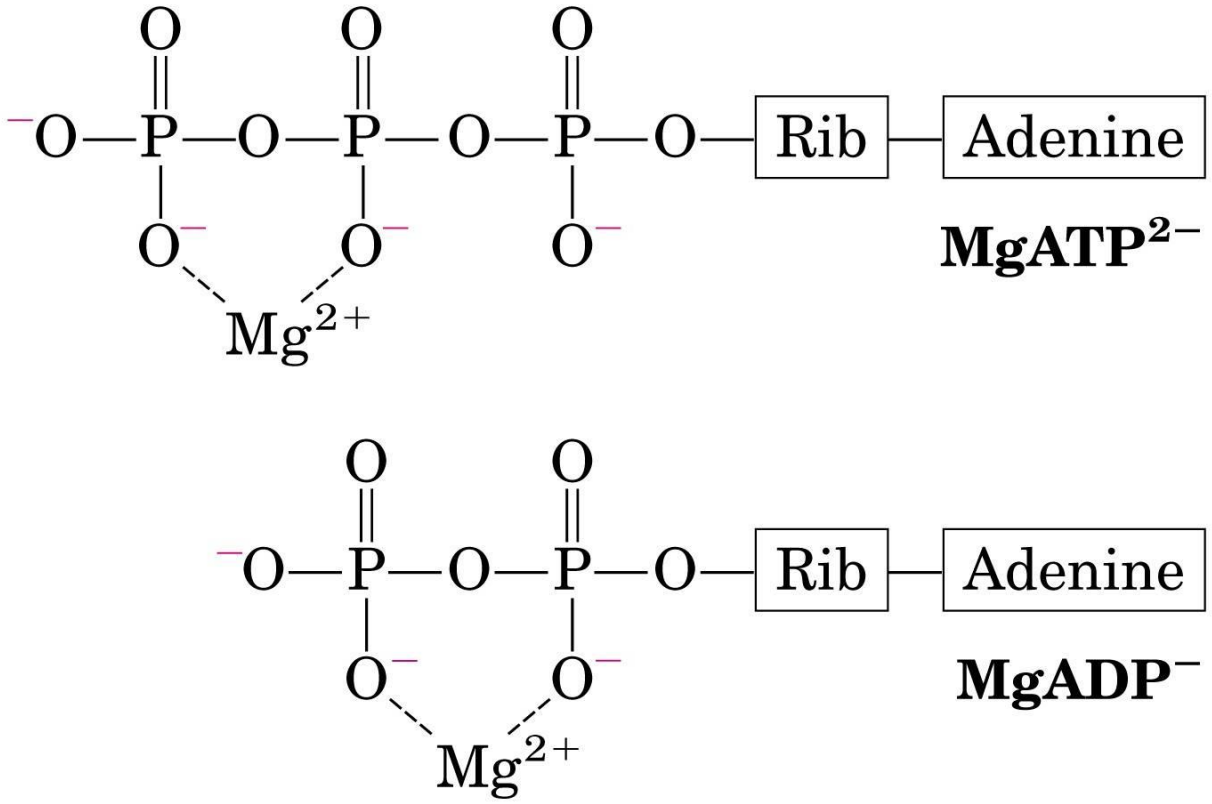


Mg^{2+} is required.

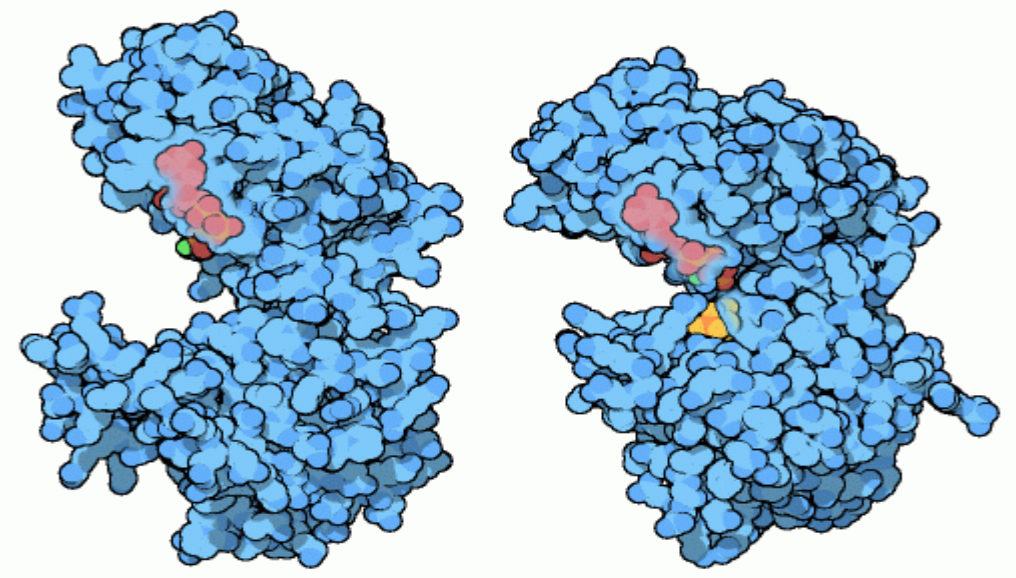
At the level of the active site, γ– phosphate of ATP is made more susceptible to nucleophilic attack by the OH^- group of the C6 of glucose.

Mg^{2+} is required.

The true substrate is the MgATP $^{2-}$ complex in which the divalent cation forms a saline bridge with the negative charges



The three-dimensional structure has shown that the enzyme is bilobed, with the active site that closes in a ring when glucose recognition occurs



This change in the conformation of the enzyme with the substrate is an example of «induced adaptation»

4 isoforms of hexokinase (I-IV) encoded by four different genes with specific tissue expression are described:

In the muscle, hexokinase I, II, III are expressed.

In the liver, hexokinase IV, (called glucokinase) is expressed.

Hexokinase IV have particular kinetic and regulatory characteristics.

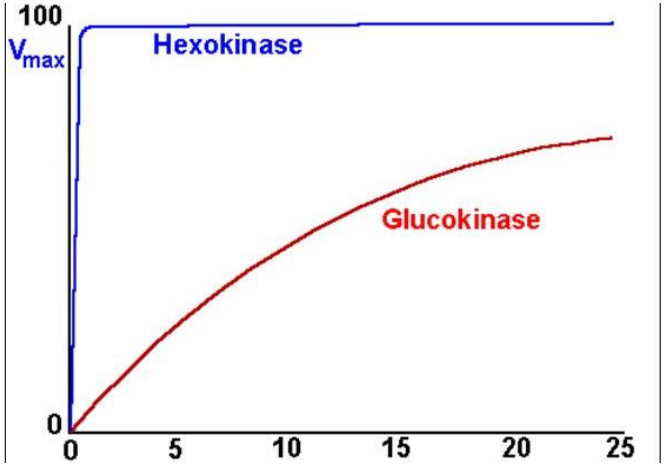
Hexokinase preferably uses glucose as an acceptor of the phosphate group, but in other tissues it can also use other hexoses such as
D-fructose or D-mannose.

The different forms of hexokinase present in the liver and muscle reflect the different role these organs play in carbohydrate metabolism:

- Muscle uses glucose to produce energy:
- it needs a source of glucose that can be quickly used during anaerobic oxidation following muscle contraction.
- Rapid ATP production

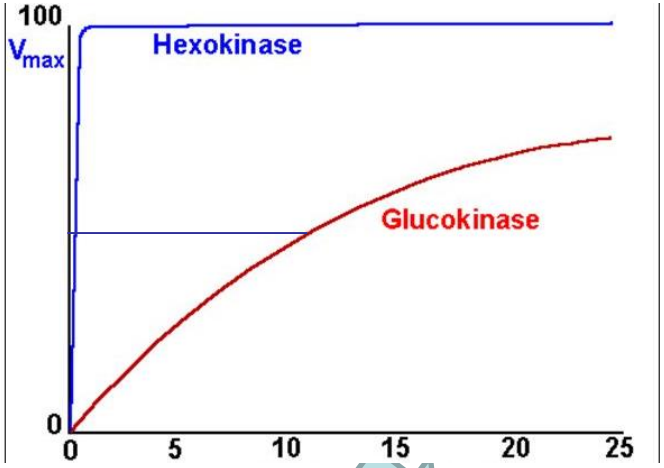
Liver maintains a constant blood glucose concentration:

- producing and exporting glucose to different tissues based on individual needs
- - removing and storing glucose as glycogen when there is an excessive intake from the diet



The role of glucose in the liver is to regolate blood sugar.
.

While in the muscle it is to produce energy



High affinity for glucose: since the glucose that enters the myocytes from the blood is sufficient to saturate the enzyme,
it always works at maximum speed

Km
Muscle is low

Km
Liver is high

Lower affinity for glucose.
the glucose concentration at which the enzyme is half saturated is higher than the normal concentration of glucose in the blood.

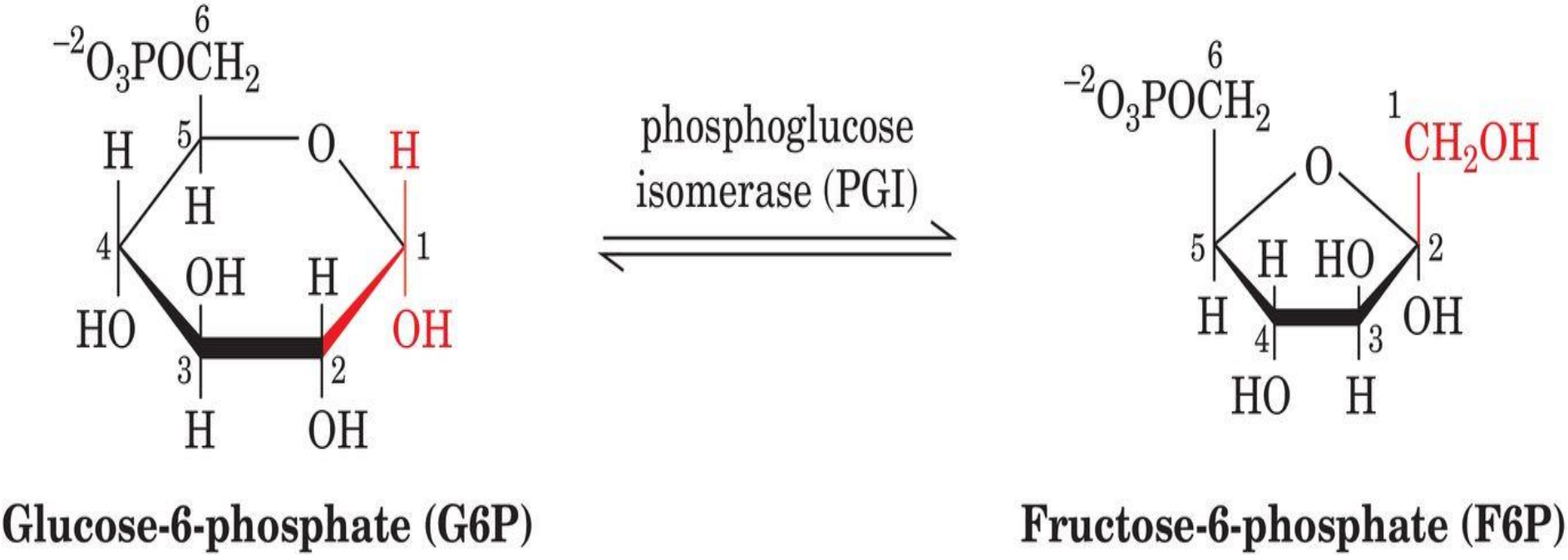
Few concentration of glucose are converted to glucose 6-phoshate and sent towards glycolysis.

When concentration of glucose exceeds blood concentration (5mM), glucose sent towards glycolysis

Reaction 2: Reversible isomerization of an aldose to ketose

Conversion of Glucose-6-phosphates (G6P), to fructose-6-phosphates (F6P), by phosphohexose isomerase (PGI= also called PhosphoGlucose Isomerase)

PGI converts reversibly glucose in fructose



Glucose-6-phosphate (G6P)

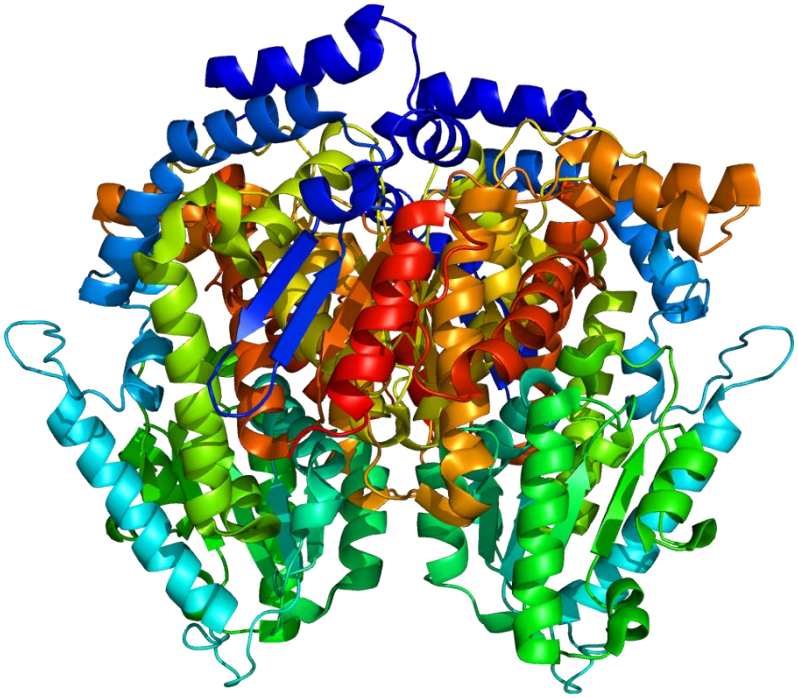
Fructose-6-phosphate (F6P)

$\Delta G'^{\circ} =$ -1,7 kJ/mole

Isomerization of the aldose Glucose 6-phosphate gives the ketose: Fructose-6-phoshphate.

Reversible isomerization of an aldose to ketose

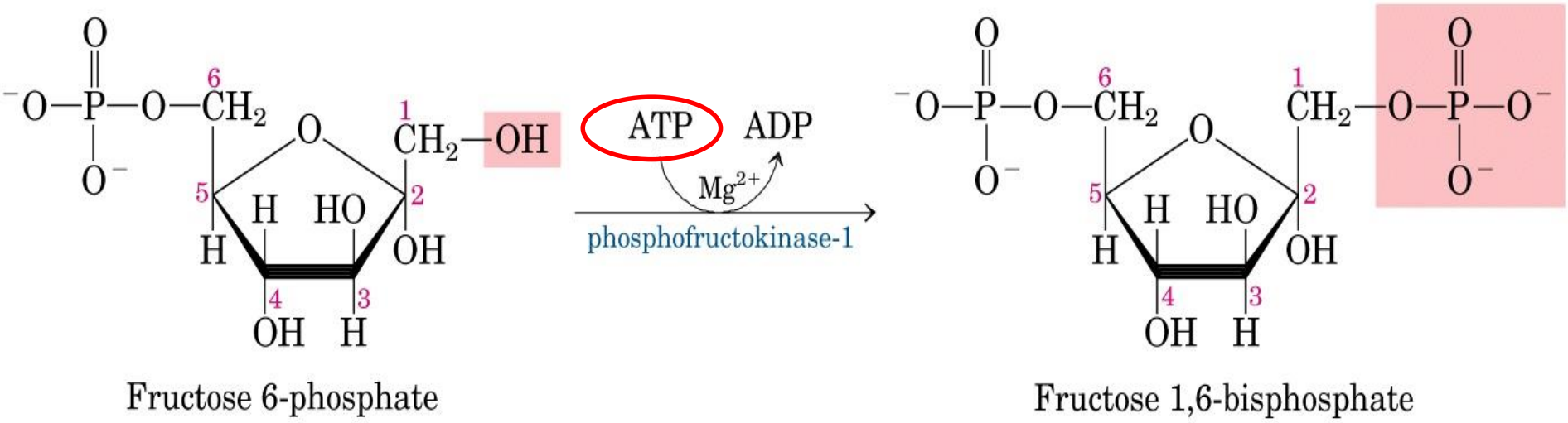
Phosphohexose isomerase (PGI= also called PhosphoGlucose Isomerase)



Reaction 3: Second ATP Utilization

PHOPHORYLATION

Fructose-6-phosphate is again phosphorilated (by ATP) on C-1 and forms Fructose-1,6-bisphosphate by phosphofructokinase-1(PFK-1).



Fructose 6-phosphate

Fructose 1,6-bisphosphate

This is an irreversible reaction

$\Delta G'^{\circ} = -14.2$ kJ/mol

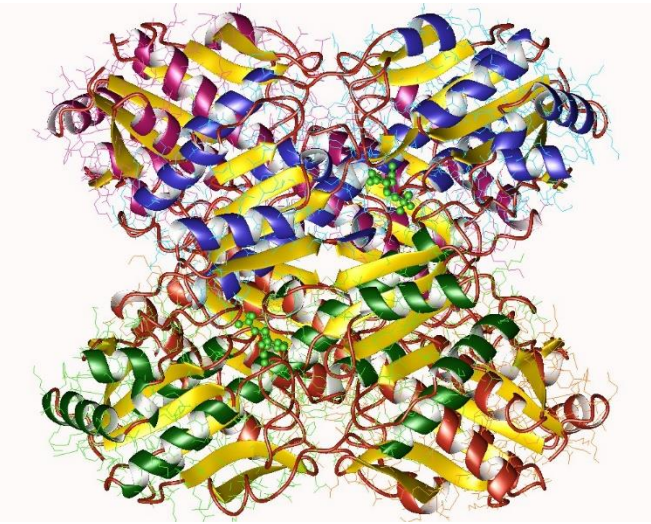
catalyzed by phosphofructokinase enzyme.

Phosphofructokinase-1(PFK-1).

The enzyme is a tetramer formed by two pairs of α and β subunits

ATP is the phosphate donor and forms an ester bond with the C1 of the fructose 6 phosphate.

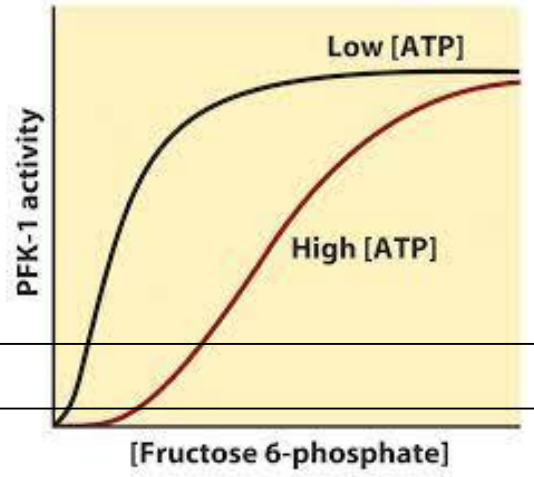
Requires Mg2+



It is a stage of regulation

It is inhibited by high intracellular ATP levels.

It is triggered by high levels of ADP and AMP



Fructose-6-phosphate + ATP -> Fructose-1,6-bisphosphate + ADP

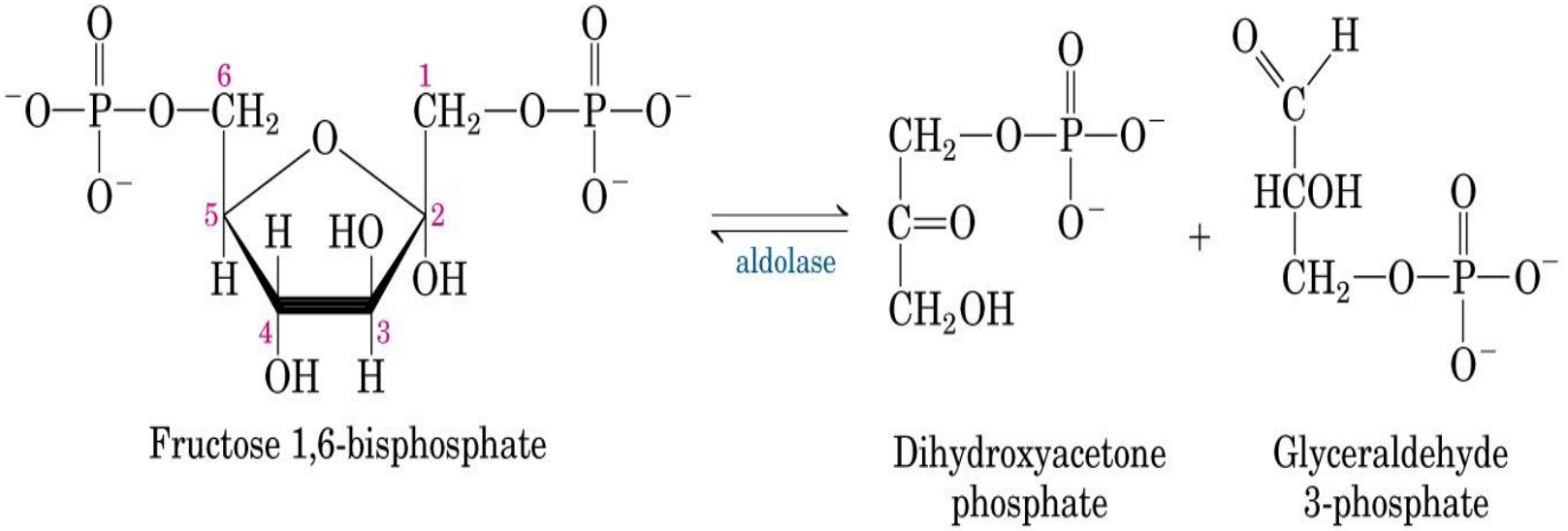
Reaction 4:

BREAKDOWN

Fructose 1,6-bisphosphate is cleaved to yield two molecules with 3 carbon atoms:

dihydroxyacetone phosphate (DHAP)

glyceraldehyde-3-phosphate (GAP)



Fructose 1,6-bisphosphate

Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

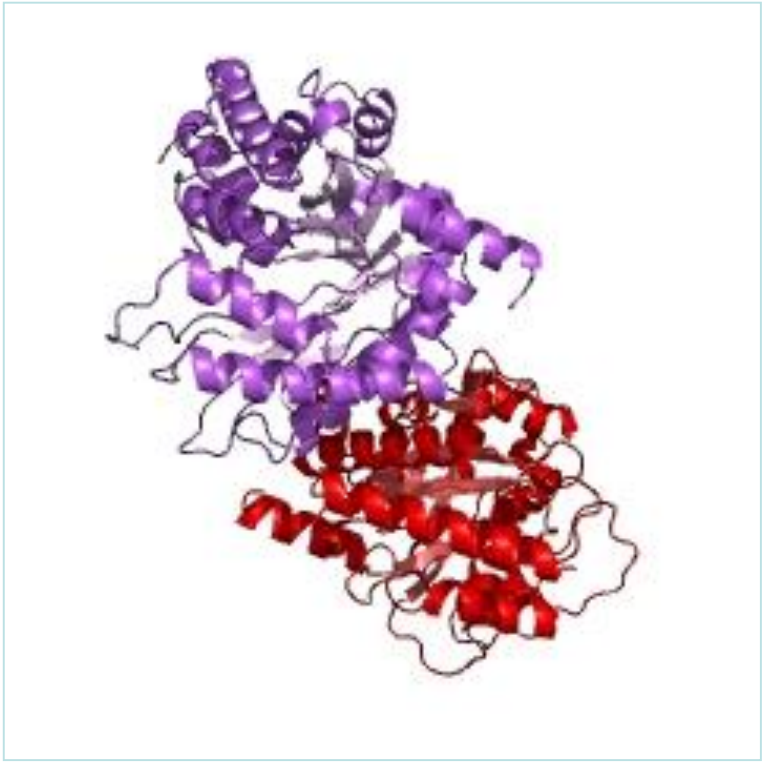
- This reaction is catalyzed by Aldolase.

$\Delta G'^{\circ} = 23.8$ kJ/mol

The reaction is Reversible

Fructose 1,6-bisphosphate aldolase is costituted by

4 identical subunits:
Each subunit contains an active site, characterized by the presence of a lysine

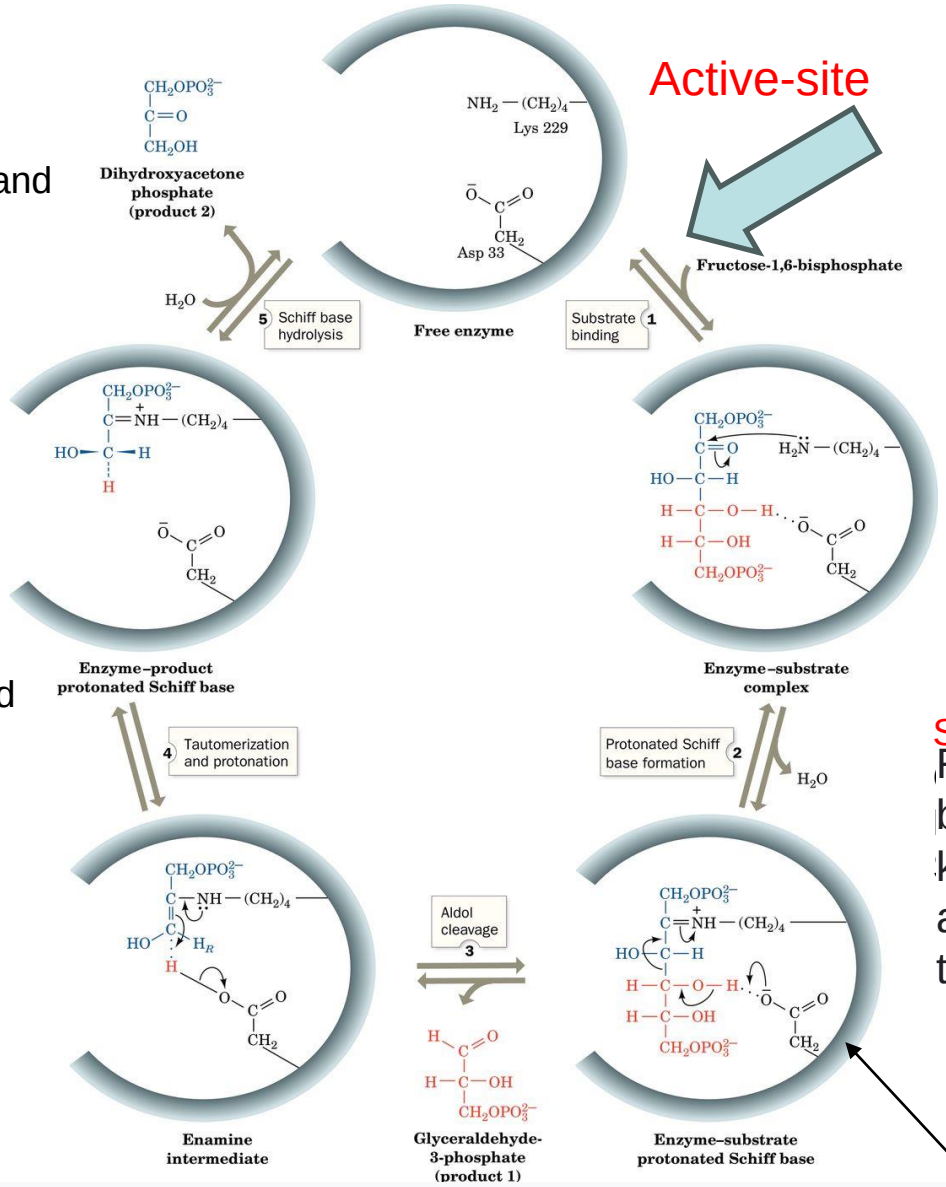


Fructose 1,6-bisphosphate -> dihydroxyacetone phosphate + glyceraldehyde-3-phosphate

Aldolase catalyzed a **aldol splitting** (the opposite of aldol condensation)

Fructose 1,6-bisphosphate -> dihydroxyacetone phosphate + glyceraldehyde-3-phosphate

STEP 5:
Schiff base hydrolyzed and released DHAP

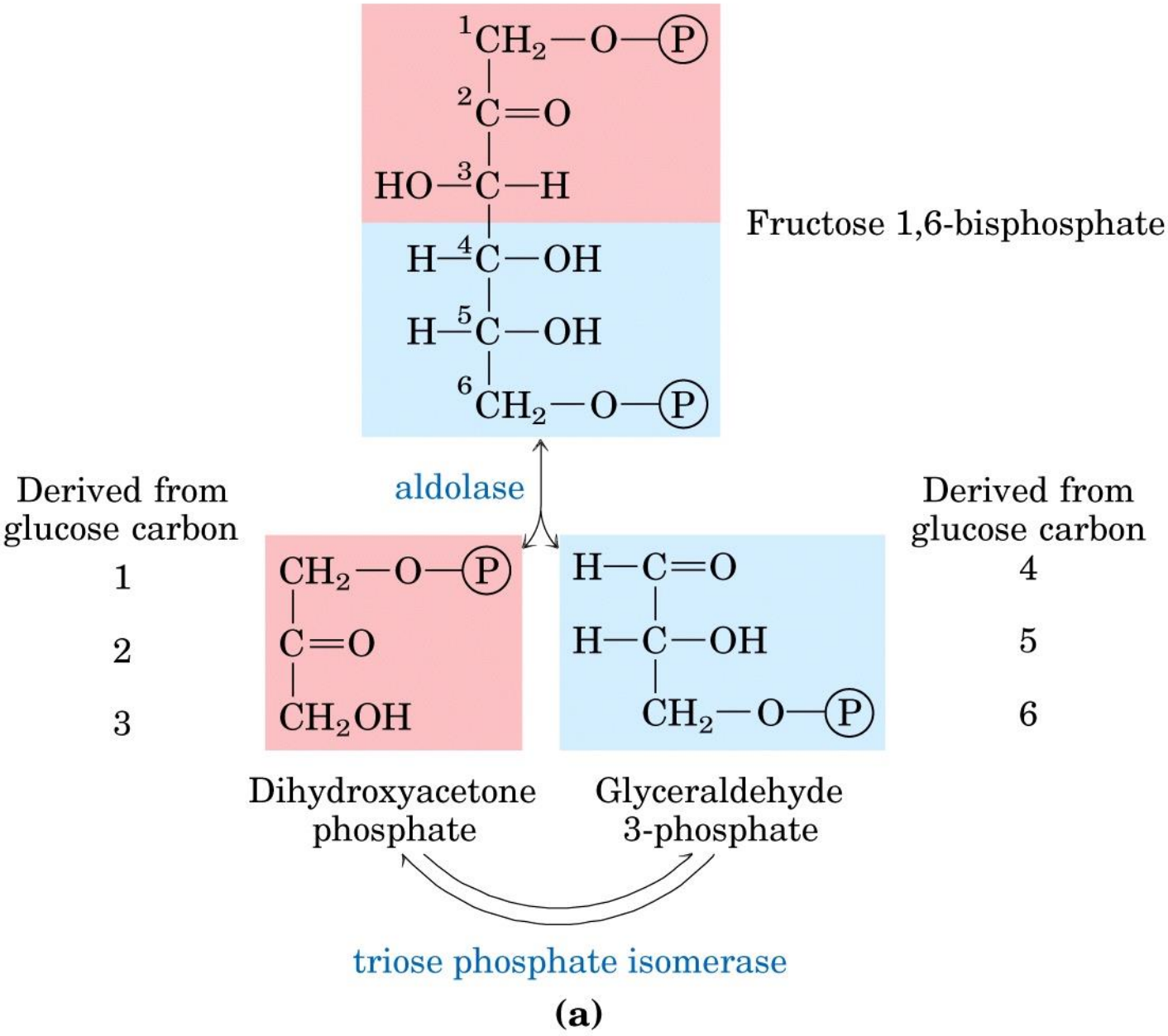


Step 1:
Binding and opening the ring
Fructose 1,6 bisphosphates binds the active site

STEP 4:
Protonated Schiff base and tautomerization

Step 2:
Formation of the Schiff base between the C2 of the ketose and the epsylon-amino group of the lysine of the enzyme

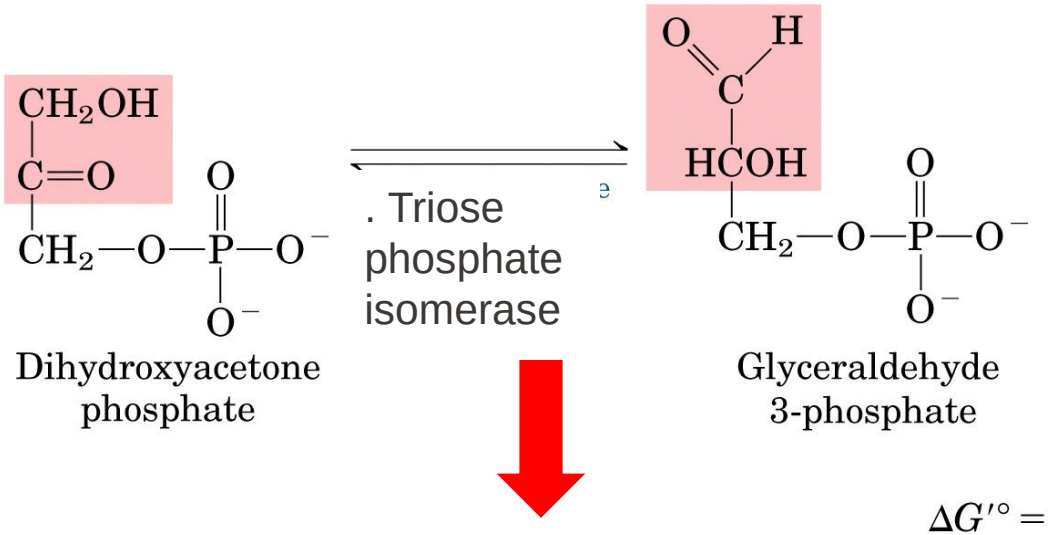
STEP 3:
Asp residue leads to the breaking of the bond between C3 and 4 and the glyceraldehyde 3 phosphate is released.


Fructose 1,6-bisphosphate
aldolase
Derived from glucose carbon
1
2
3
Derived from glucose carbon
4
5
6
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
triose phosphate isomerase

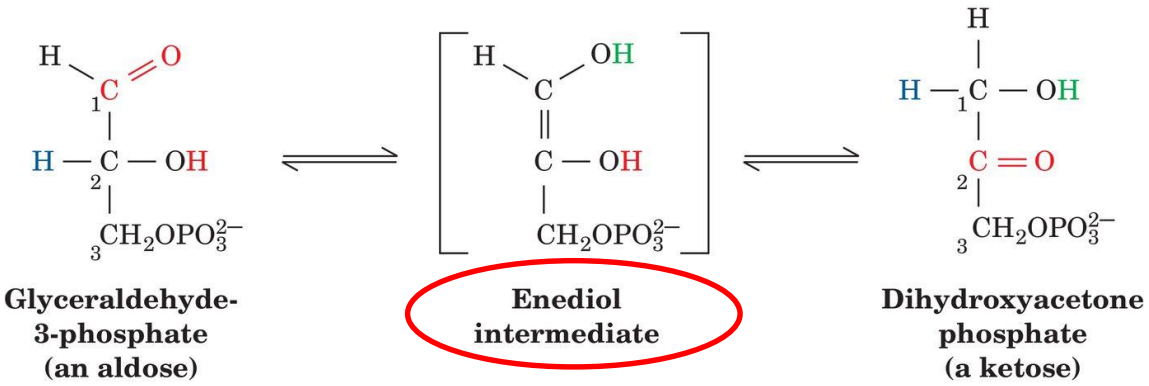
(a)

Reaction 5

Dihydroxyacetone phosphate is oxidized to form Glyceraldehyde -3-phosphate by triose phosphate isomerase (TIM)



$\Delta G'^{\circ} = 7.5$ kJ/mol

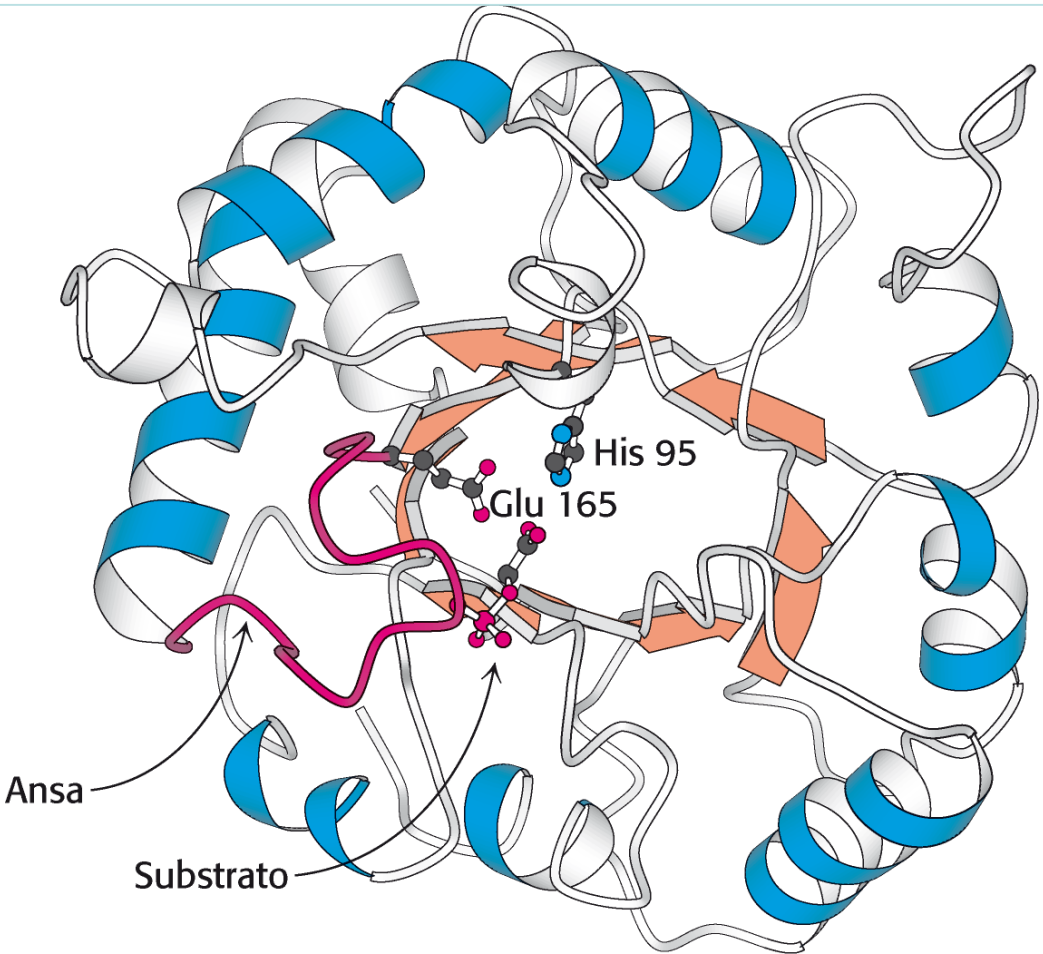


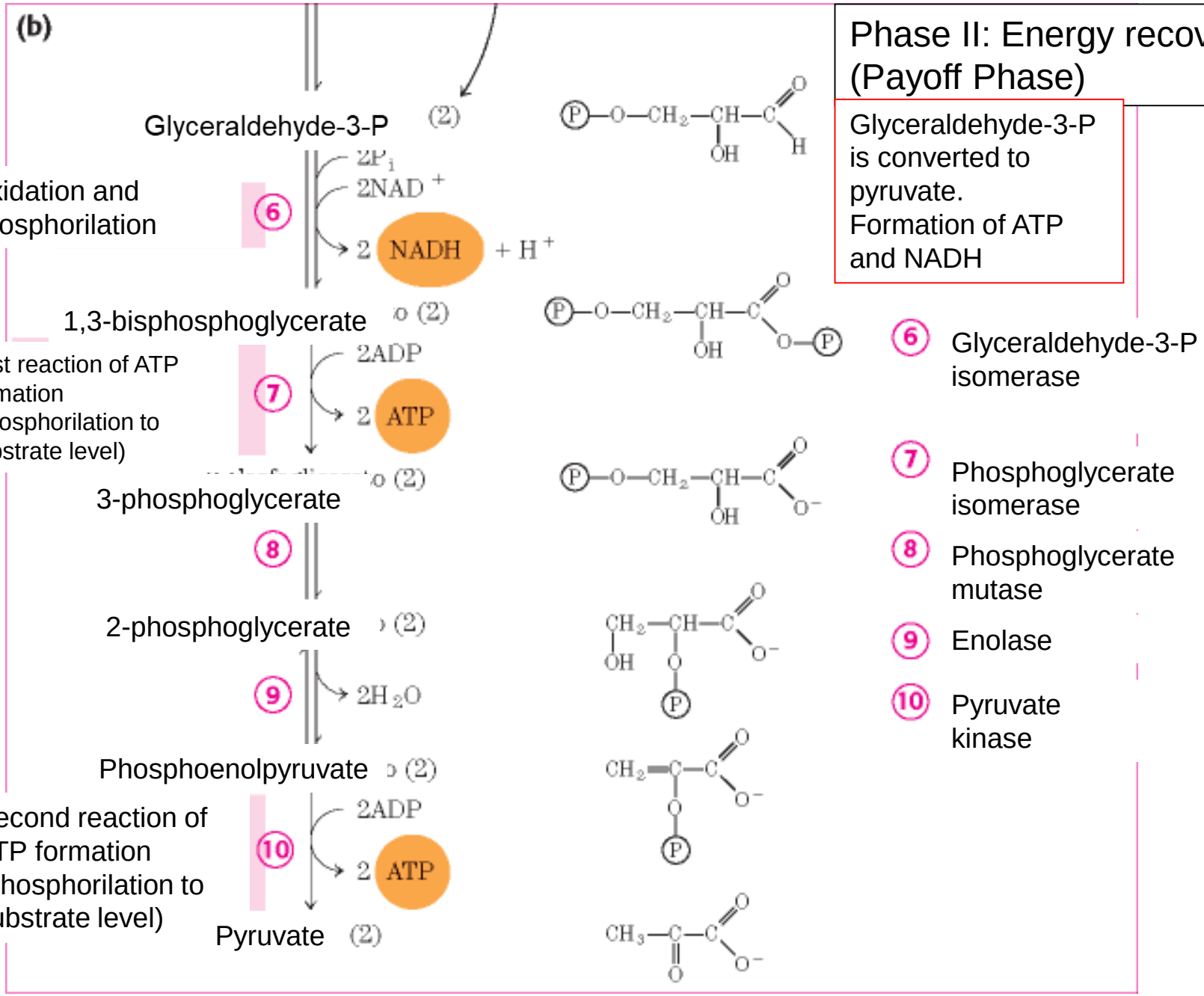
Glycolysis continues to double stechiometria

Triose phosphate isomerase

Triose phosphate isomerase is a dimer composed of identical subunits,

Active site of the enzyme contains a glutamate residue, involved in the catalytic mechanism.





Oxidation and phosphorilation

First reaction of ATP formation (phosphorilation to substrate level)

Second reaction of ATP formation (phosphorilation to substrate level)

Phase II: Energy recovery (Payoff Phase)

Glyceraldehyde-3-P is converted to pyruvate.
Formation of ATP and NADH

6 Glyceraldehyde-3-P isomerase

7 Phosphoglycerate isomerase

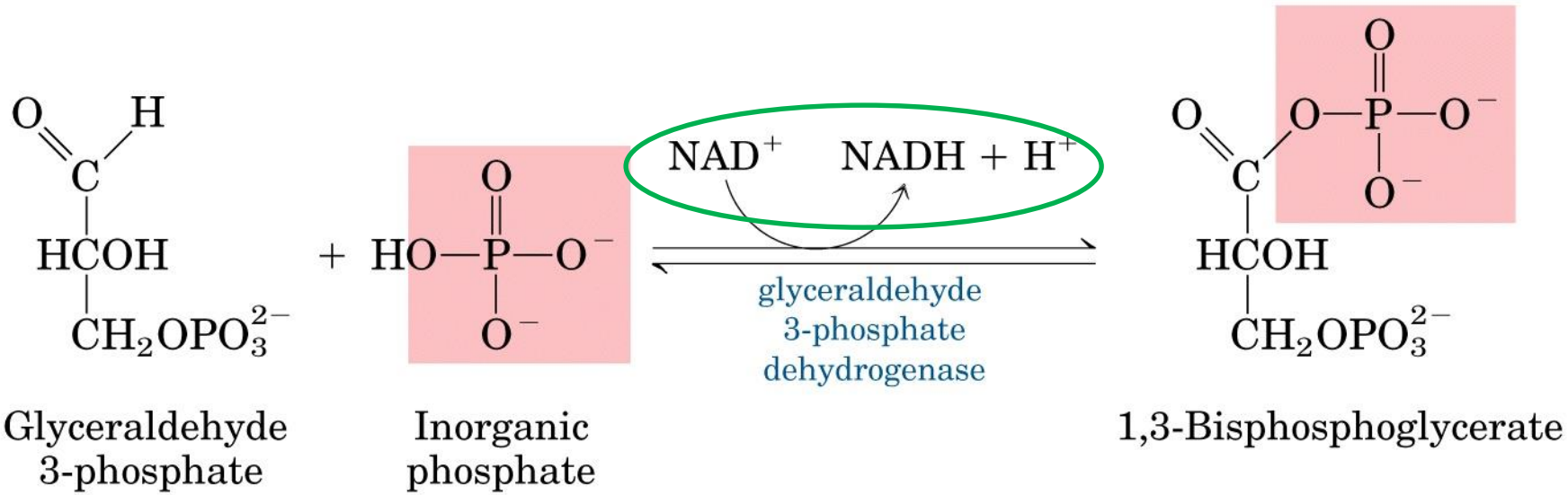
8 Phosphoglycerate mutase

9 Enolase

10 Pyruvate kinase

Reaction 6: Oxidation and Phosphorilation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate

Aldehydic group is oxidized to acyl and reacts with phosphate to forms acyl phosphate

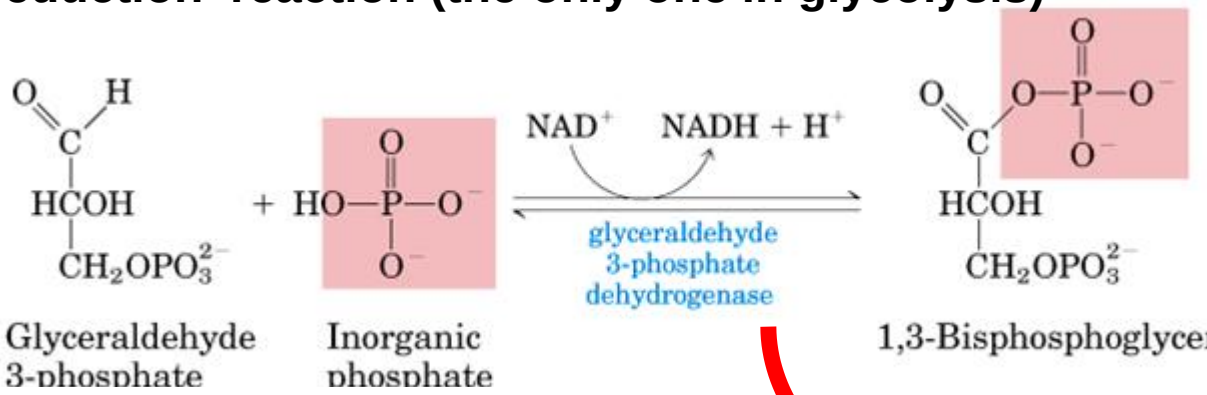


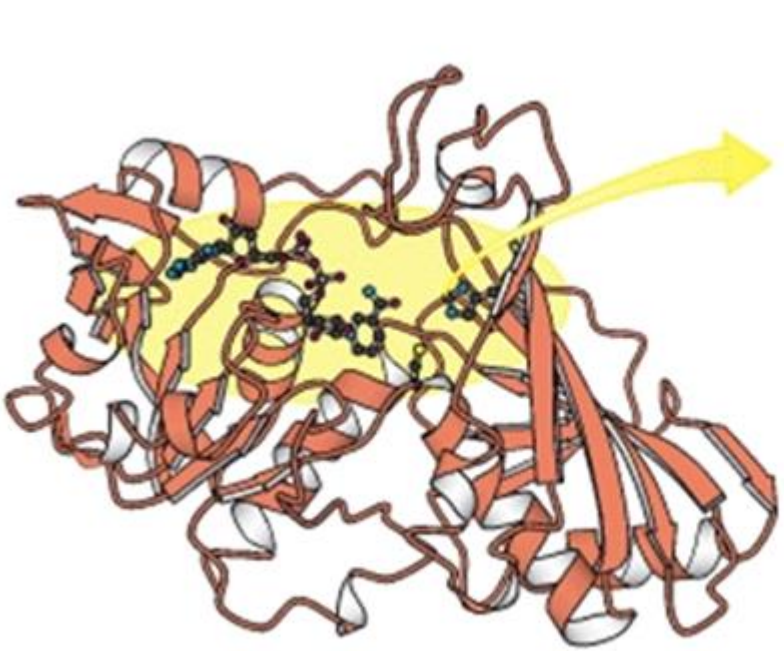
$2NAD^+ + 2Pi \rightarrow 2NADH + 2H^+$

$\Delta G'^\circ = 6.3$ kJ/mol

- 2 molecules of Glyceraldehyde-3-phosphate are oxidized.
-

Oxidation-reduction reaction (the only one in glycolysis)





OXDOREDUCTASE

NAD^+ dependent

Monomeric Structure of Human Glyceraldehyde 3-Phosphate Dehydrogenase

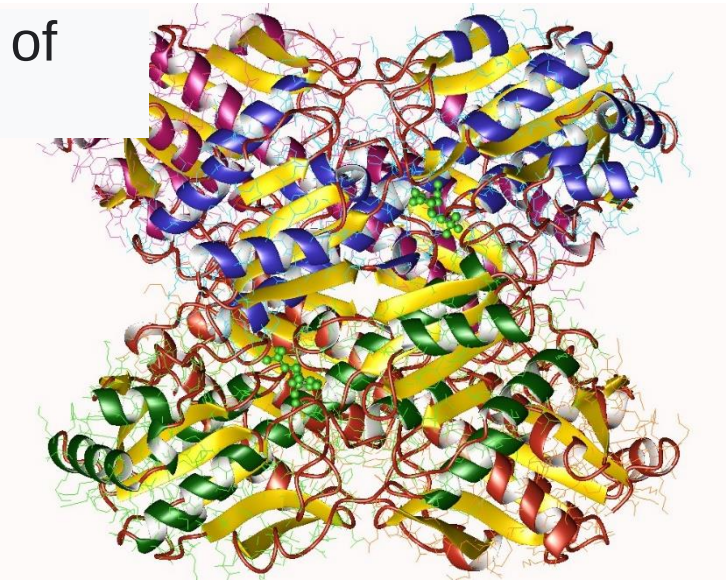
glyceraldehyde 3-phosphate ->1,3-bisphosphoglycerate

Glyceraldehyde 3-Phosphate Dehydrogenase Is a tetramer with four identical subunits.

Each subunits has a catalytic site that binds 1 molecule of NAD +

It uses NAD + as an oxidant and requires organic phosphate

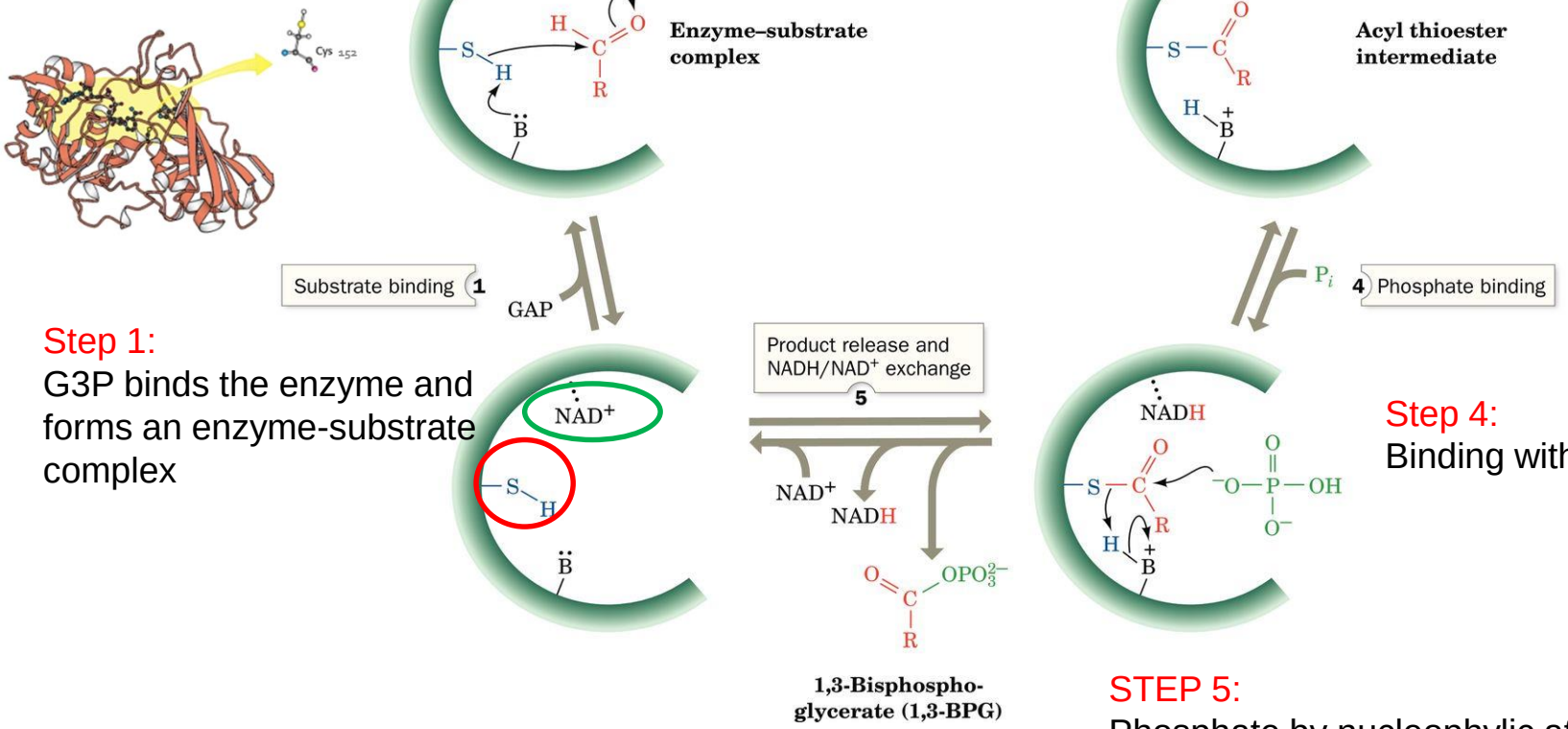
The mechanism involves a cysteine residue of the enzyme to which the substrate is bound,



Process Diagram: GAPDH Mechanism

Step 2:
-SH group by nucleophylic attack on aldehyde and form Covalent thiohemiacetal

Step 3:
Thiohemiacetal is oxydized to acyl-thioester by transfering to hydryde ion to NAD+ (storage energy)



Step 1:
G3P binds the enzyme and forms an enzyme-substrate complex

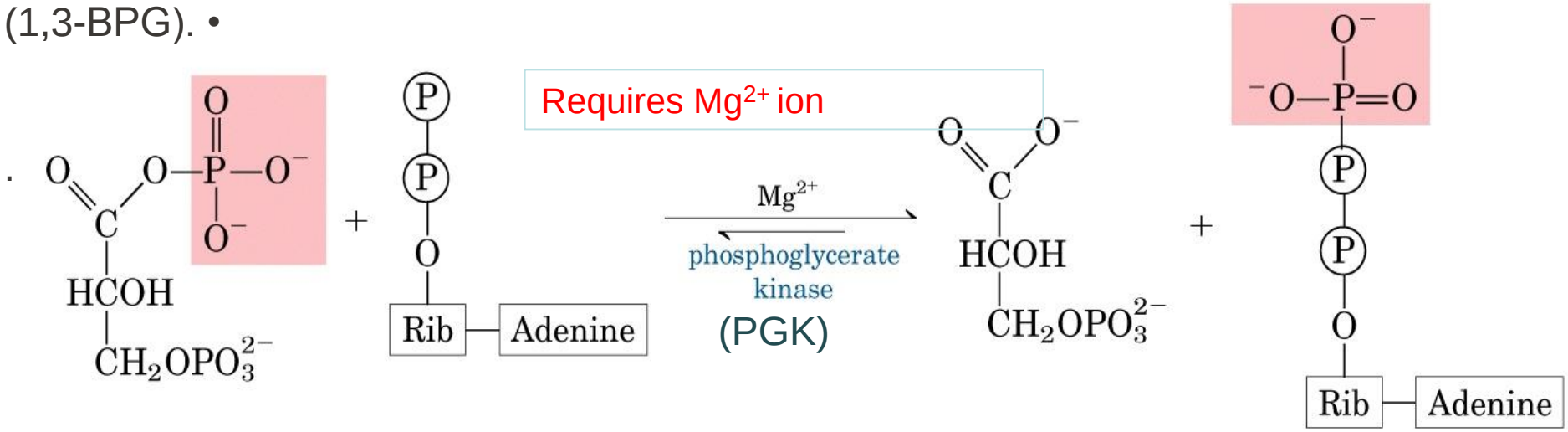
Step 4:
Binding with phosphate

STEP 5:
Phosphate by nucleophylic attack on thioester and forms 1-3BPG

Reaction 7: Substrate-level Phosphorilation

Phosphoglycerate kinase transfers the high-energy phosphoryl group from 1,3-bisphosphoglycerate to ADP that produce ATP

Addition of phosphate to ADP to give ATP is called as substrate level phosphorylation. The phosphate donor is the substrate 1,3-bisphosphoglycerate (1,3-BPG). •

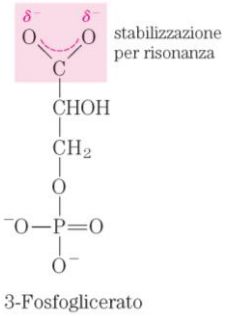


1,3-Bisphosphoglycerate ADP 3-Phosphoglycerate ATP

(1,3-BPG) (3-PG)

$\Delta G'^{\circ} = -18.5$ kJ/mol

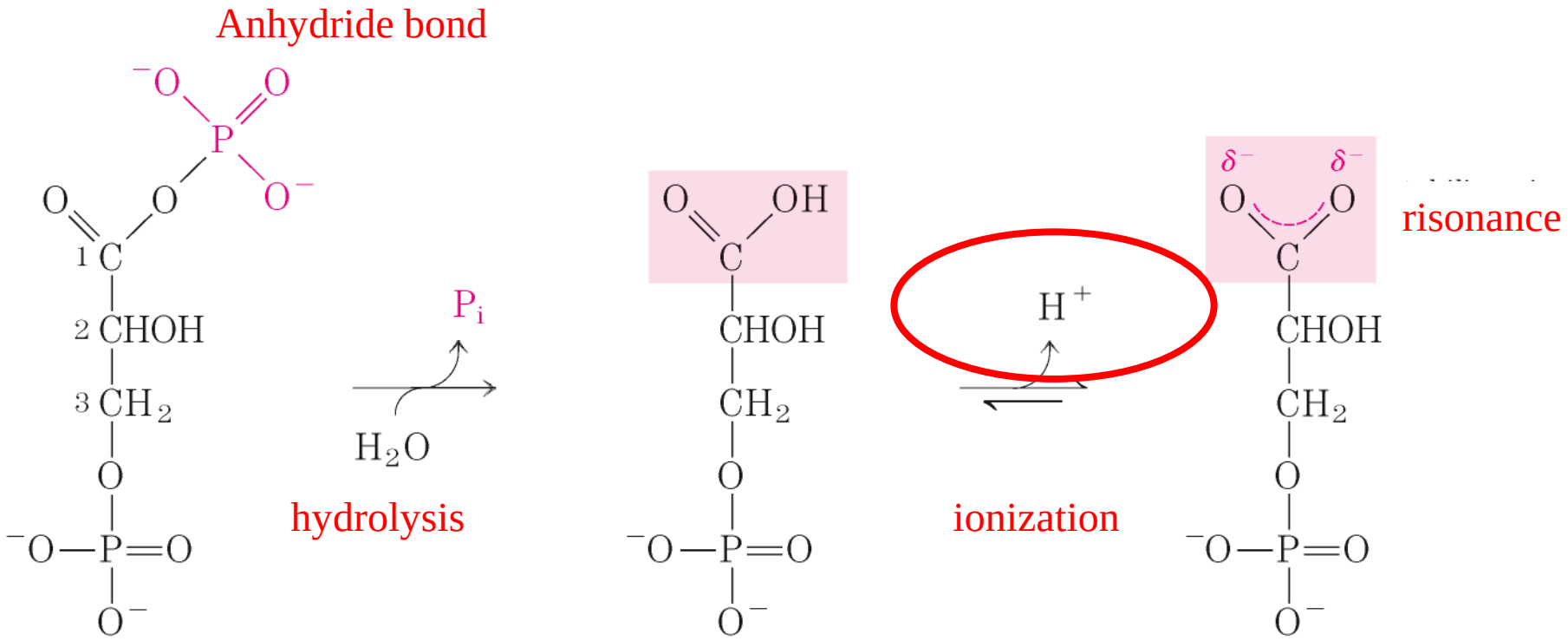
The product of this reaction is 2 molecules of 3-phosphoglycerate



FIRST SUBSTRATE LEVEL PHOSPHORYLATION

1,3 BISPHOPHOGLYCERATE (1,3-BPG)

1,3-bisphosphoglycerate contains an anhydride bond between the C-1 carboxyl group and a phosphoric acid.
Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free-energy change (ΔG= - 49.3 kJ/mol).



$$\text{1,3-Bisfosfoglicerato}^{4-} + H_2O \longrightarrow \text{3-fosfoglicerato}^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -49{,}3 \text{ kJ/mole}$$

Reaction catalyzed from glyceraldehyde 3-P dehydrogenase and Phosphoglycerate kinase are coupled (step 6 e 7):

Glyceraldehyde 3P + Pi → 1,3-bisphosphoglycerate

$\Delta G° = +6.3$ kJ / mol

1,3-bisphosphoglycerate + ADP → 3-phosphoglycerate + ATP

$\Delta G° = -18$ kJ / mol

1,3-bisphosphoglycerate + H_2O → 3-phosphoglycerate + P

$\Delta G° = -49,3$ kJ / mole

$ADP + H_3PO4 \rightarrow ATP + H_2O$ $\Delta G° = +30,5$ kJ / mole

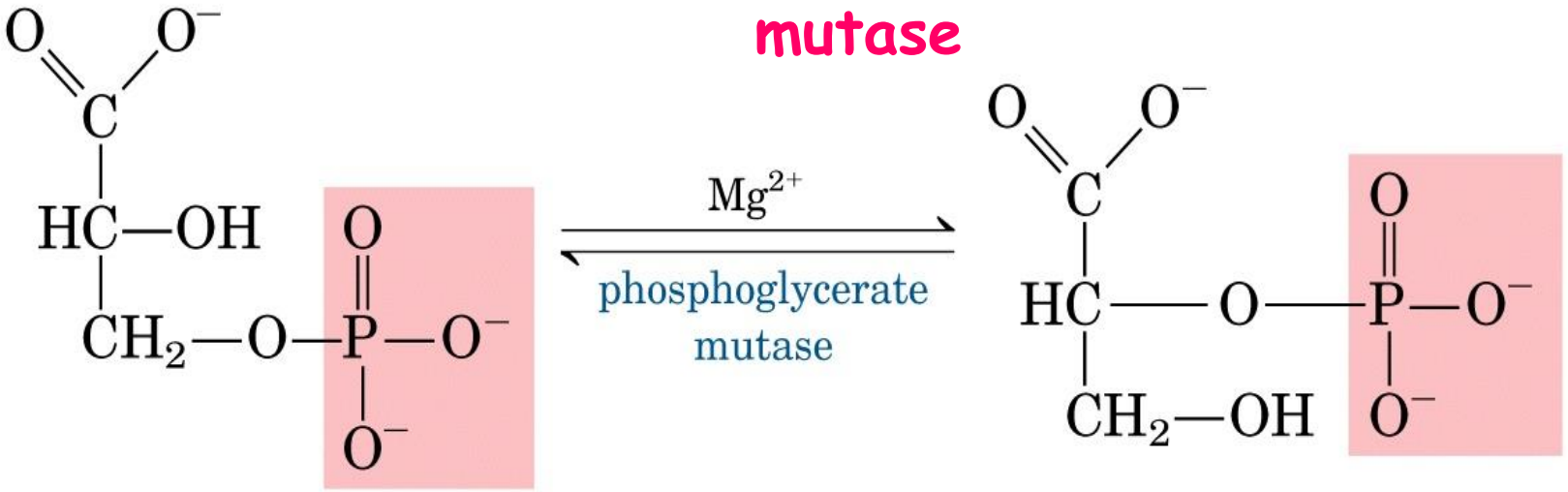
$\Delta G° = -18,8$ kJ / mole

Glyceraldehyde-3P + Pi +ADP+ NAD+ → 1,3-phosphoglycerate + ATP+ NADH+H

$\Delta G° = -12.3$ kJ / mol

Reaction 8: the remaining phosphate-ester linkage is moved **from C3 to C-2** to form 2-phosphoglycerate (2-PG)

The reversible reaction is catalyzed by phosphoglycerate mutase



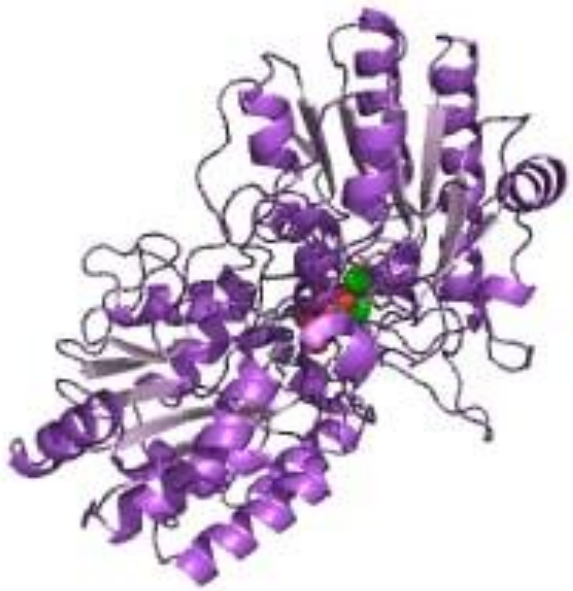
3-Phosphoglycerate

2-Phosphoglycerate

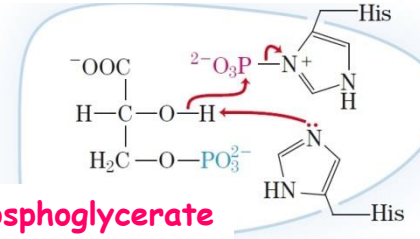
$\Delta G'^{\circ} = 4.4$ kJ/mol

low free energy of hydrolysis

…two step of Reaction



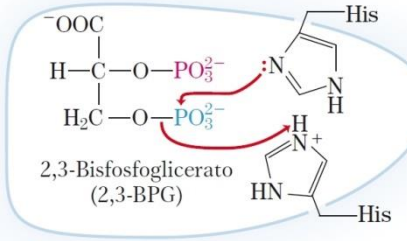
Phosphoglycerate mutase



3- phosphoglycerate

Enzyme contains two His in the active- site which one phosphorylated His (near to C2 and C3)

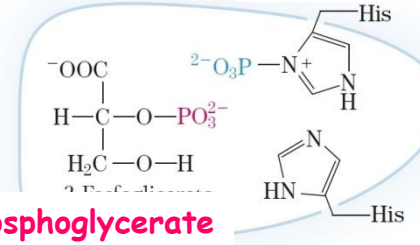
1. First residue of His transfers phosphate group to C2 and forms 2,3 BPG



Bisphosphorylated intermediate

Hb

2. The second residue of His shifts phosphate from C3 to the first residue of His

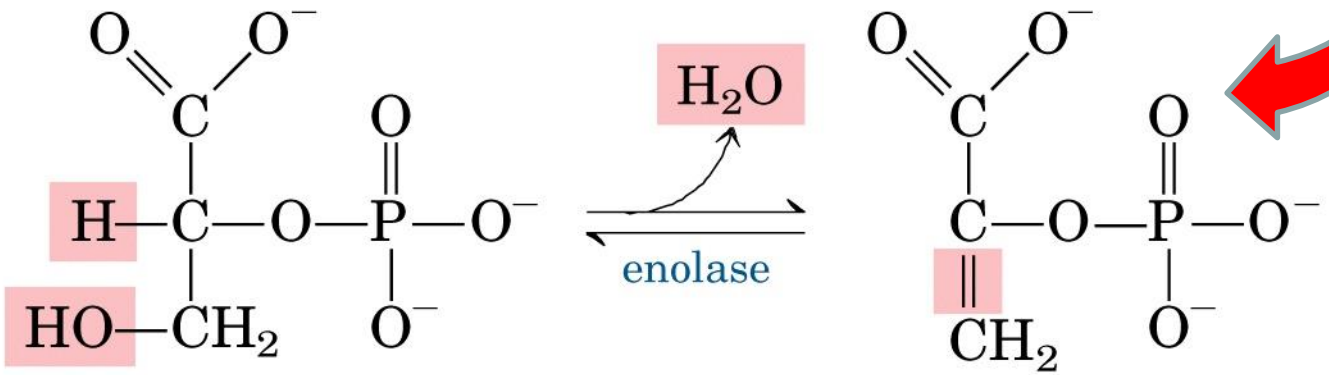


2- phosphoglycerate

Reaction 9:

DEHYDRATION OF 2-PG

The 2-phosphoglycerate is dehydrated by the action of enolase to phosphoenolpyruvate(PEP).



2-Phosphoglycerate

Phosphoenolpyruvate

(PEP)

$\Delta G'^{\circ} = 7.5$ kJ/mol

1. Loss of an H atom from C2 and an OH group from C3
2. H_2O is release
3. A double bond is formed between C2 and C3

• This is the second reaction in glycolysis where a high-energy phosphate compound is formed. •

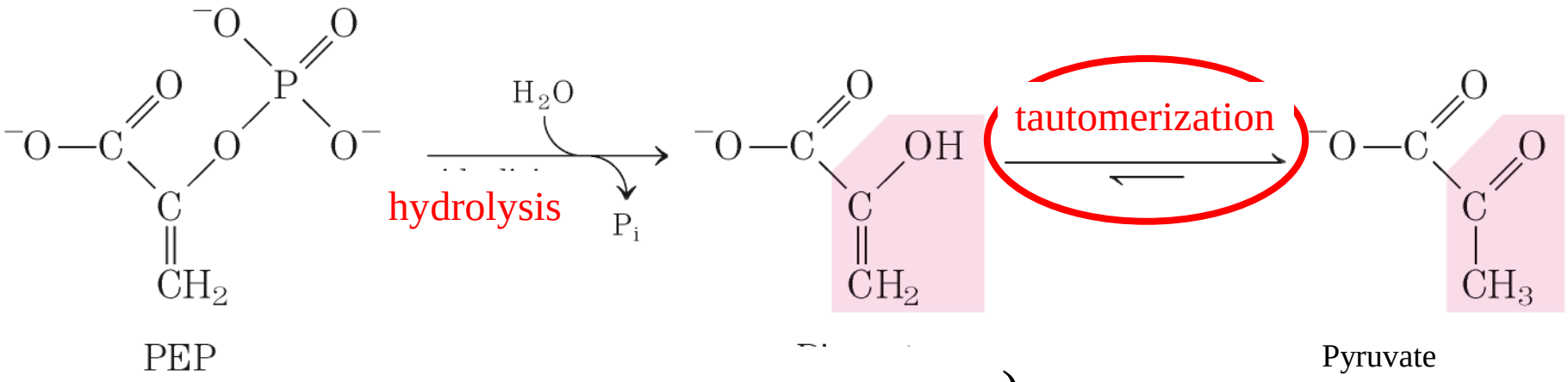
This compound is the phosphate ester of the enol tautomer of pyruvate.

This is a reversible reaction.

ΔG°' (High)

Phosphorylated Compounds have Large Free Energies of Hydrolysis

Phosphoenolpyruvate (PEP) contains a phosphate ester bond that undergoes hydrolysis to yield the enol form of pyruvate, and this direct product can tautomerize to the more stable keto form. Because the reactant (PEP) has only one form (enol) and the product (pyruvate) has two possible forms, the product is stabilized respect to the reactant.
This is the greatest contributing factor to the high standard free energy of hydrolysis of phosphoenolpyruvate: ΔG= -61.9 kJ/mol



PEP

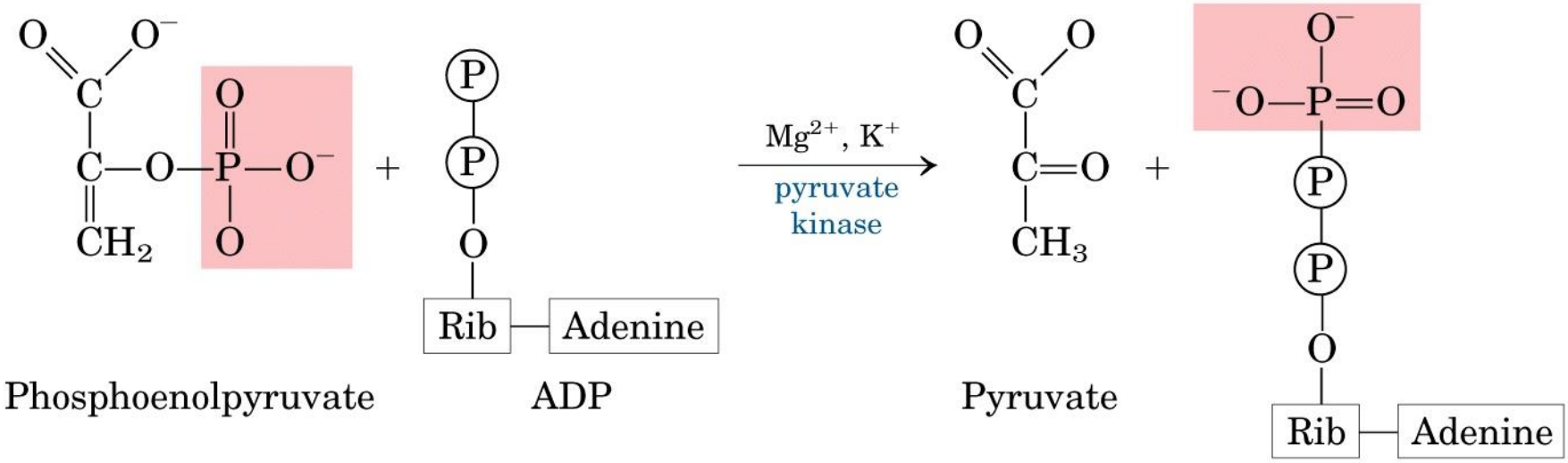
Pyruvate (Enolic form)

Pyruvate (Ketonic form)

$$PEP^{3-} + H_2O \longrightarrow piruvato^{-} + P_i^{2-}$$
$$\Delta G'^{\circ} = -61{,}9 \text{ kJ/mole}$$

Reaction 10: TRANSFER OF PHOSPHATE FROM PEP to ADP

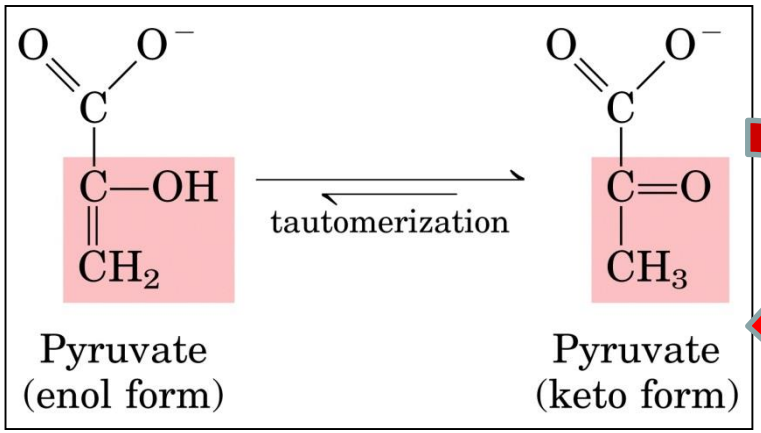
It is a transfer of high energy phosphoryl group from PEP to ADP (to form ATP)



Phosphoenolpyruvate ADP Pyruvate ATP

This is a non-oxidative phosphorylation reaction.

Second phosphorilation at substrate level



$\Delta G'^{\circ} = -31.4$ kJ/mol
exergonic

PYRUVATE KINASE

phosphoenolpyruvate + ADP → pyruvate + ATP

$\Delta G^{\circ\prime}$ = -31,4 kJ / mole

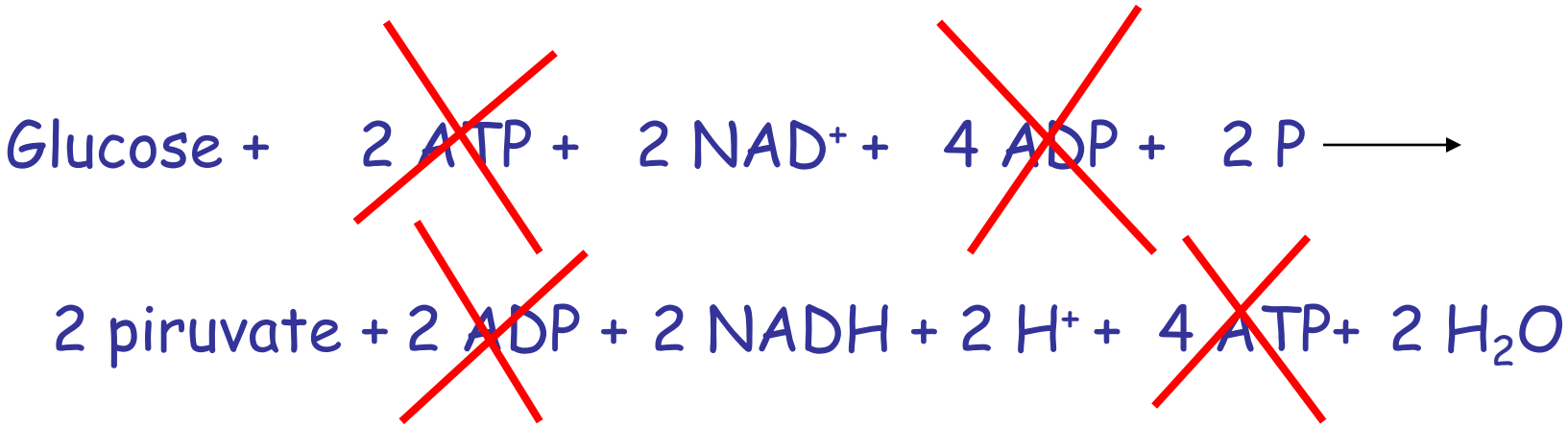
1) Phosphoenolpyruvate + H_2O → pyruvate + H_3PO_4

ΔG° = -61,9 kJ / mole

2) ADP + H_3PO_4 → ATP + H_2O

ΔG° = +30,5 kJ / mole

Balance of glycolysis



The total reactions of glycolysis can be summarized as:

$$Glucose + 2NAD^+ + 2\ ADP + 2P \longrightarrow 2Pyruvate + 2NADH + 2\ H^+ + 2ATP + 2\ H_2O$$

-85kJ/mole

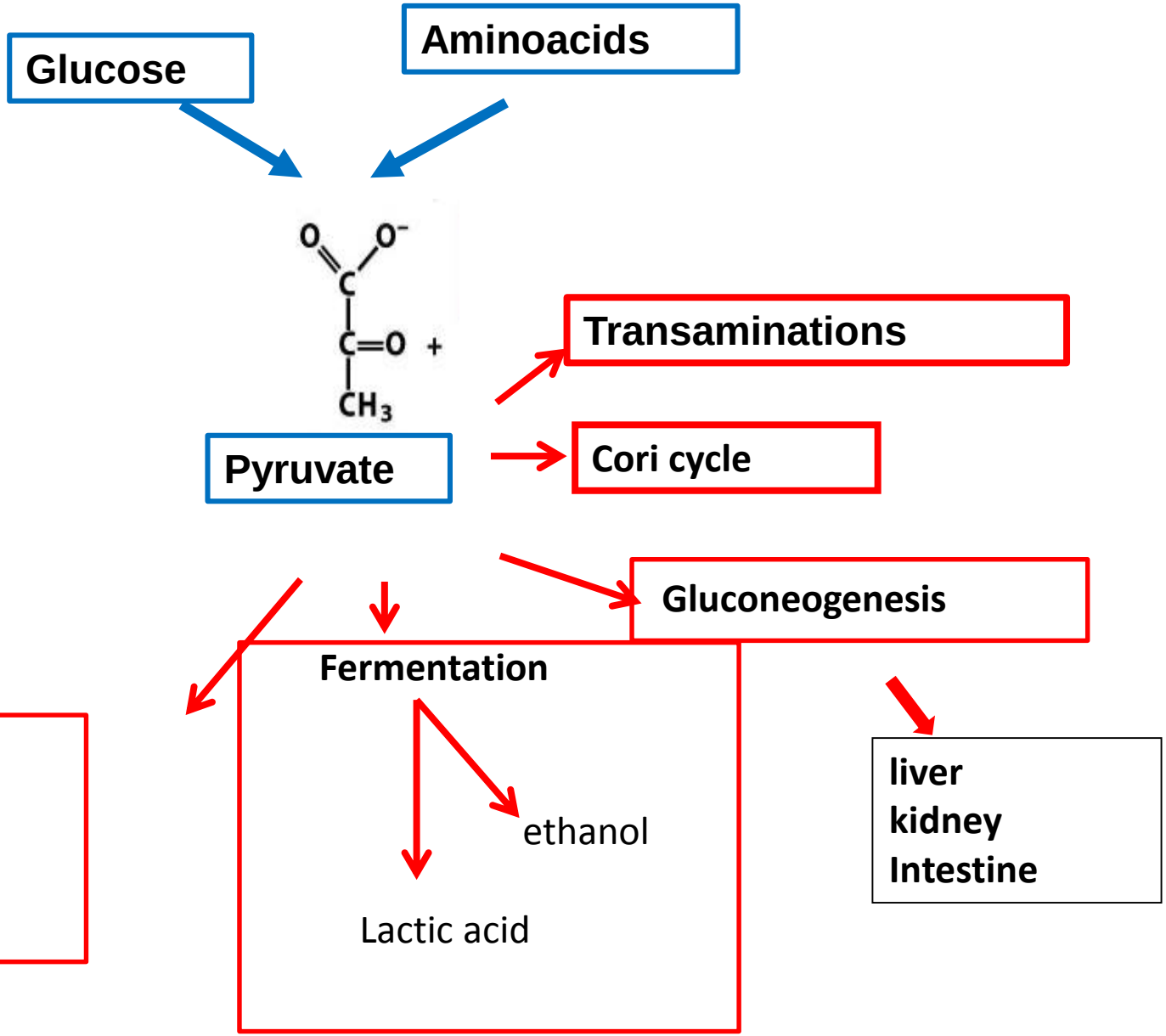
Two molecules of NADH formed in citosol during glycolis are oxydated in aerobic conditions by transfer their electrons to respiratory chain localize to mitochondria

.

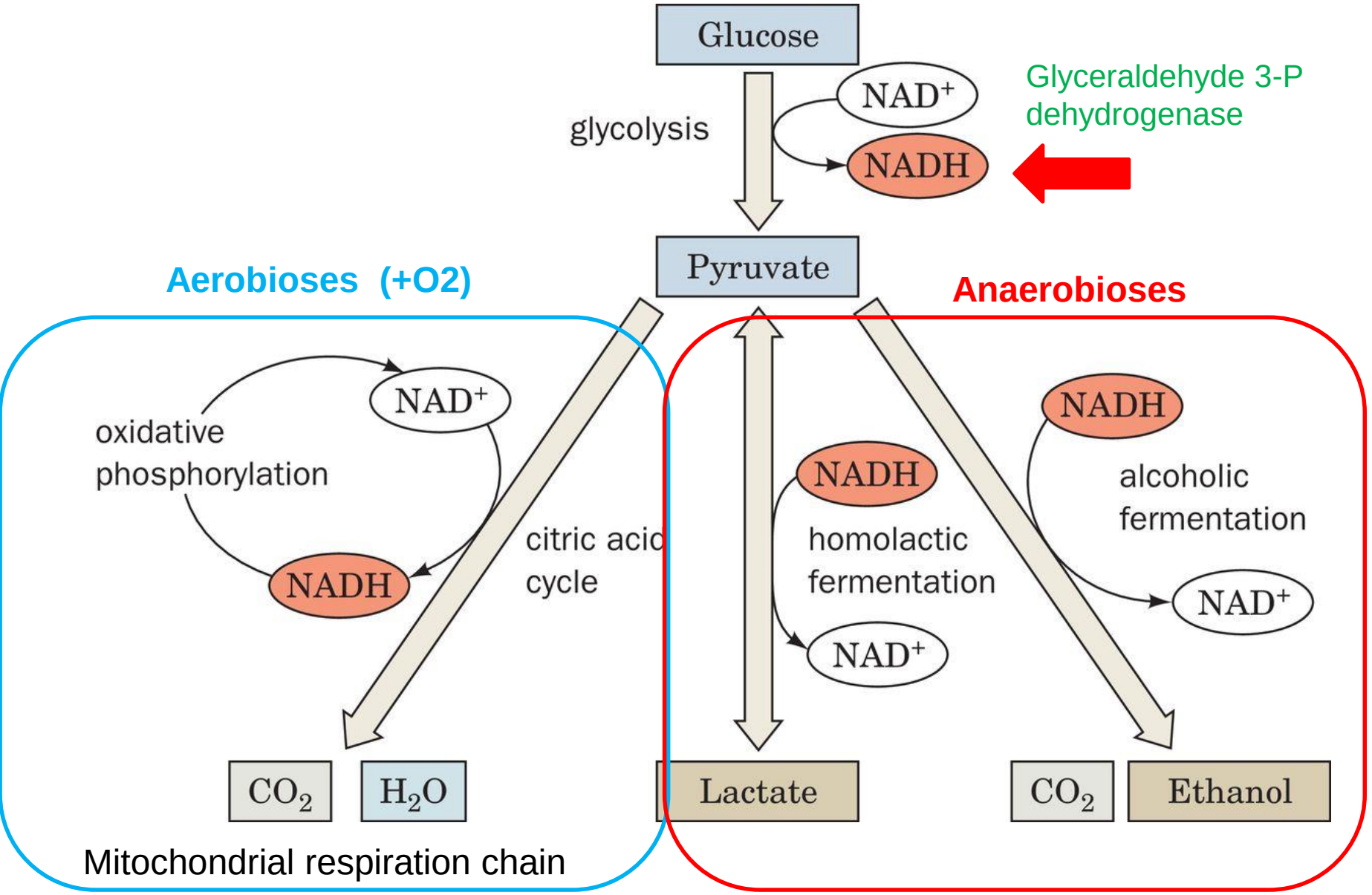
Last electrons is oxygen:

$$2NADH + 2H^+ + O_2 \longrightarrow 2NAD^+ + H_2O$$

Pyruvate: intermediate of several metabolic reactions



Metabolic Fate of Pyruvate

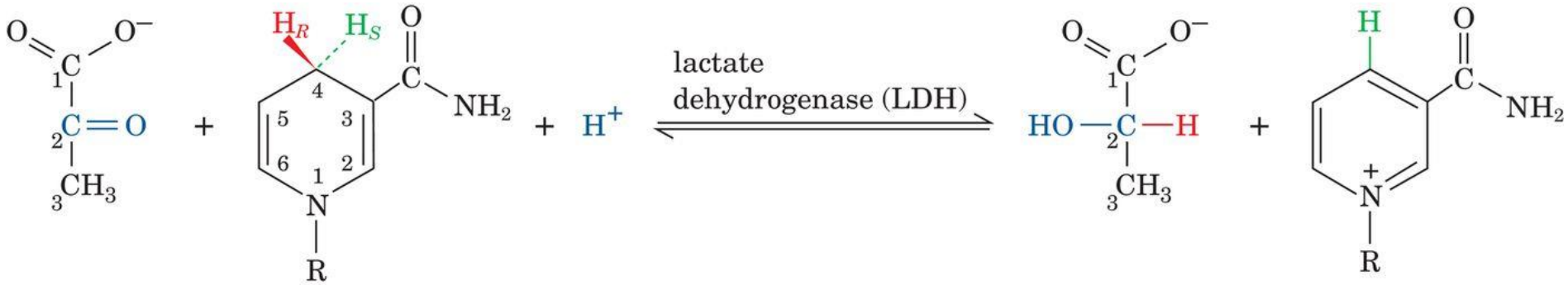


1. Homolactic Fermentation

In muscle, particularly during vigorous activity when the demand for ATP is high and oxygen has been depleted (in cells with few mitochondria like ERYTRHOCYTES), ATP is obtained by anaerobic glycolysis.

LACTATE DEHYDROGENASE catalyzes the oxidation of NADH by piruvate to yeald NAD+ and lactate

This reaction is often classified as Reaction 11 of glycolysis



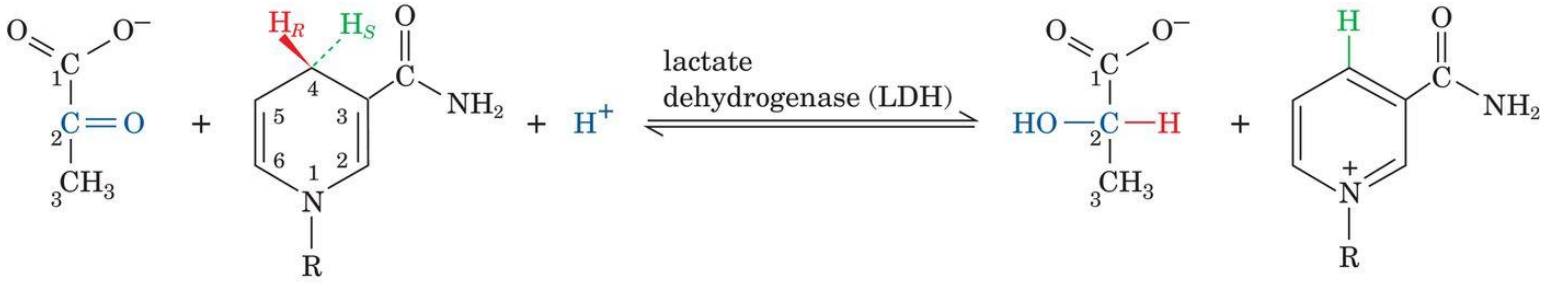
Pyruvate **NADH** **L-Lactate** **NAD+**

Hydrogen at C4 of NADH is transferred to the pyruvate at C2 to form L-Lactate

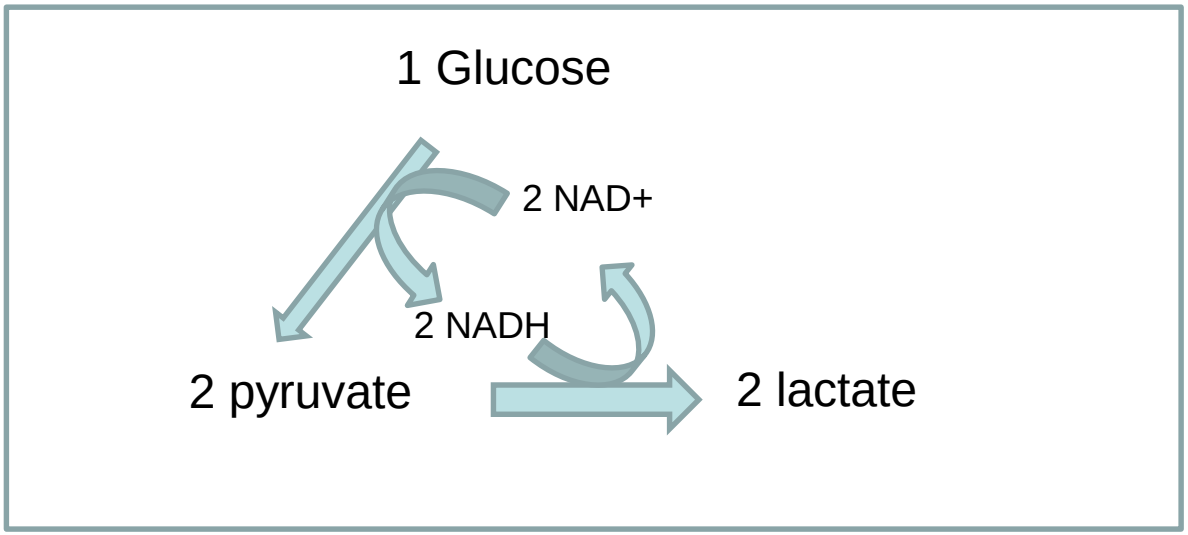
NAD+ is regenerated by GAPDH (glyceraldehyde-3P-dehygrogenase)

Lactate may be transfer out of the cell, bring to liver and converted to glucose

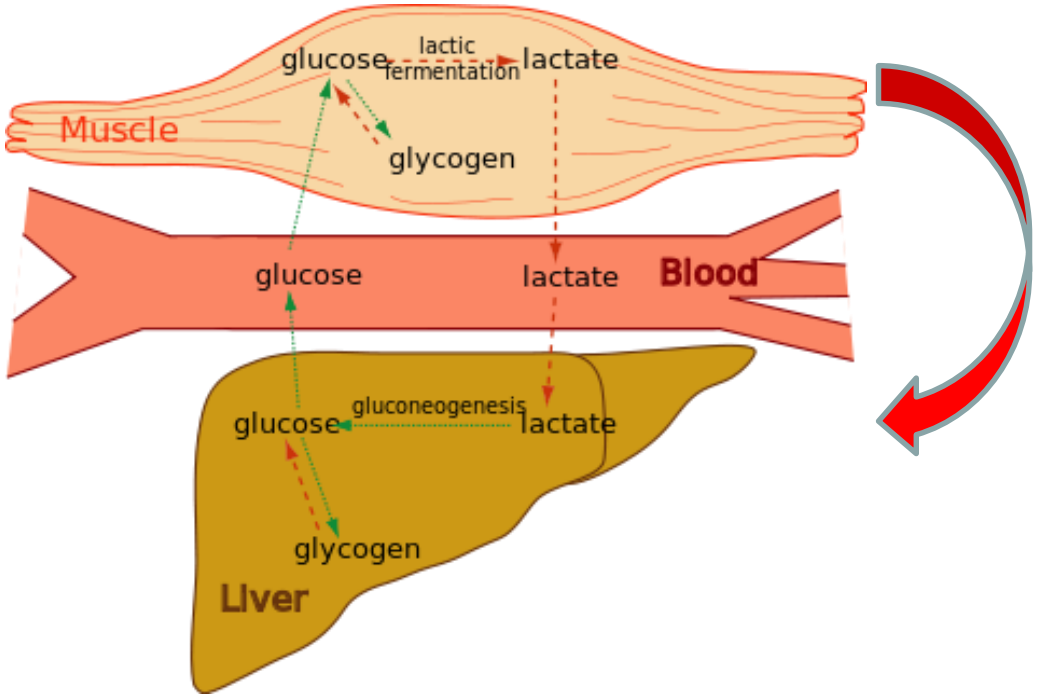
Glycolysis produces two molecules of glyceraldehyde 3-P (from 1 molecule of glucose), two molecules of pyruvate and two molecules of NADH



2 **Pyruvate** 2 **NADH** 2 **L-Lactate** 2 **NAD$^+$**

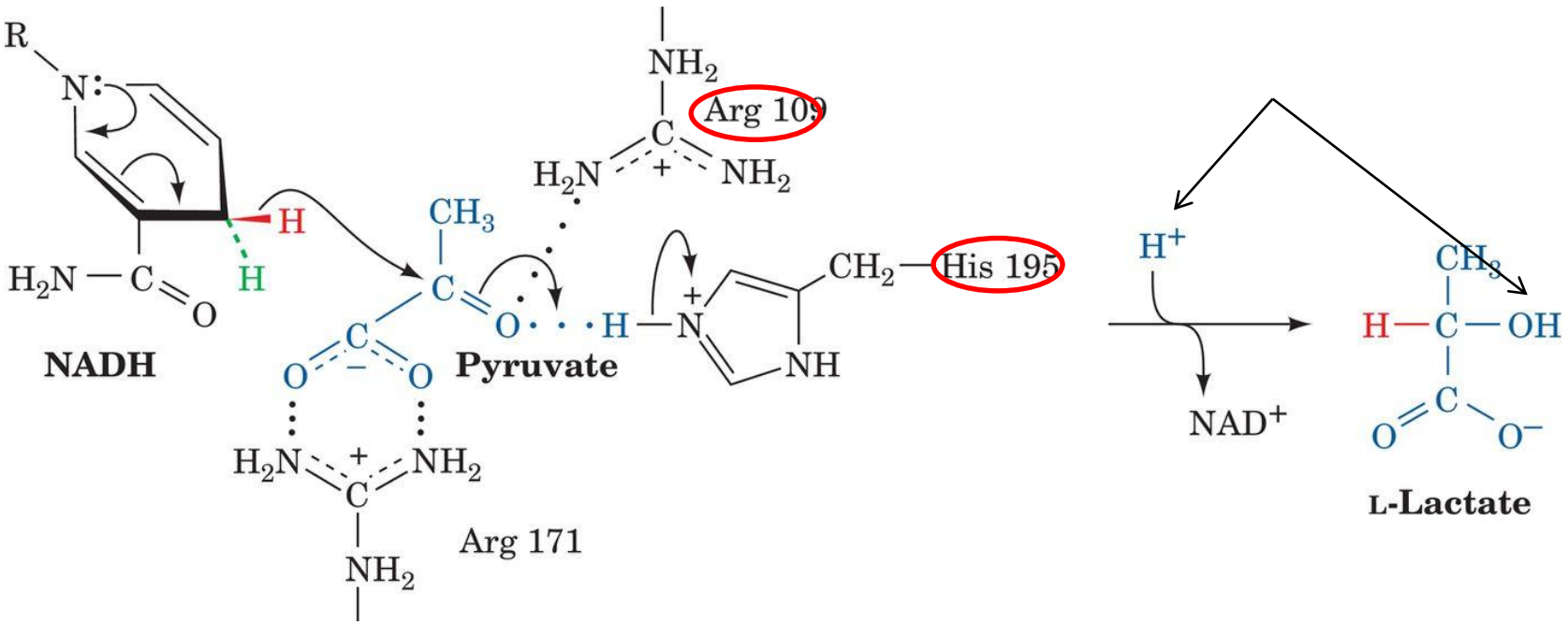


Cori Circle



Lactate formed (skeletal muscle) can be recycled. Carried in the blood to the liver and converted to glucose during the recovery from strenuous muscle

REACTION Mechanism OF LDH



The reaction involves direct hydride transfer from NADH to pyruvate
Imidazolium group of His 195 donates a proton to the pyruvate carbonyl oxygen atom.

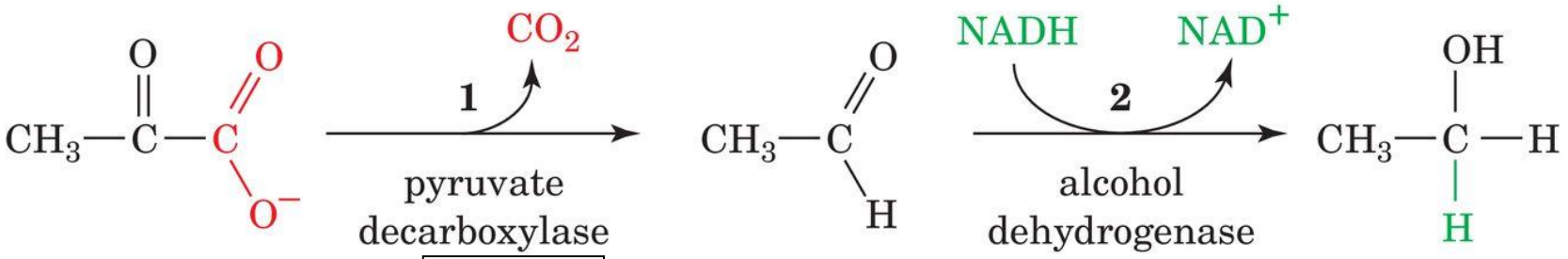
The latter process is facilitated by the positive charge on the nearby side chain of Arg 109.

2 Alcoholic Fermentation

Yeast produces ethanol and CO_2 via two consecutive reactions

Intestinal bacteria (in the absence of oxygen) can metabolize pyruvate by alcholic fermentation

Used by glyceraldehyde 3-P dehydrogenase



PDC (enzyme not present in animals)

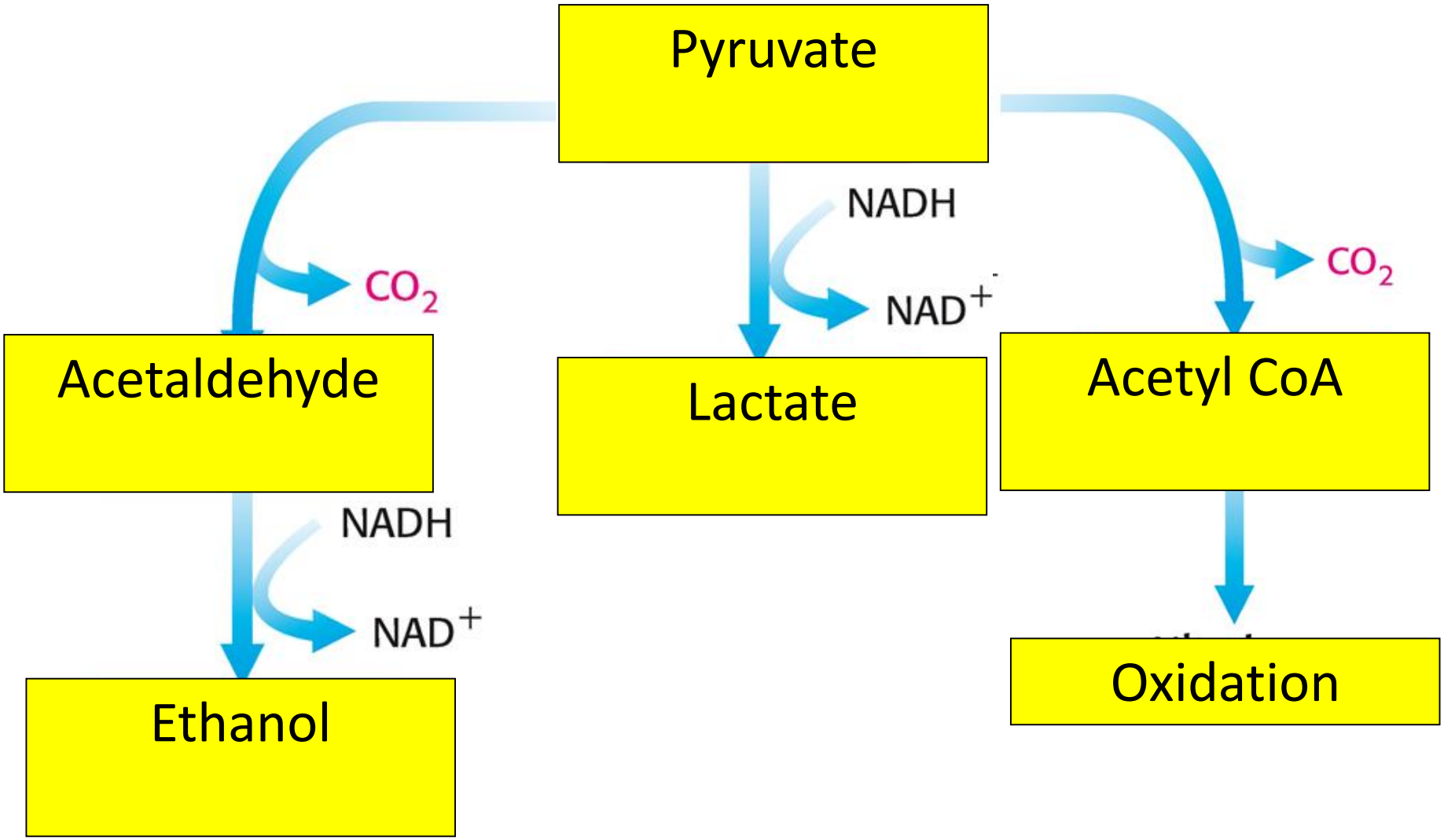
First reaction

Decarboxylation of pyruvate to form acethaldehyde and CO_2

Second reaction

Acetaldheyde is reduced to ethanol by NADH in a reaction catalyzed by alcohol dehydrogenase (ADH)

Coenzyme: thiamine pyrophosphates (TPP) active (thiazolium ring)


Pyruvate
CO_2
NADH
NAD^+
CO_2
Acetaldehyde
Lactate
Acetyl CoA
NADH
NAD^+
Ethanol
Oxidation

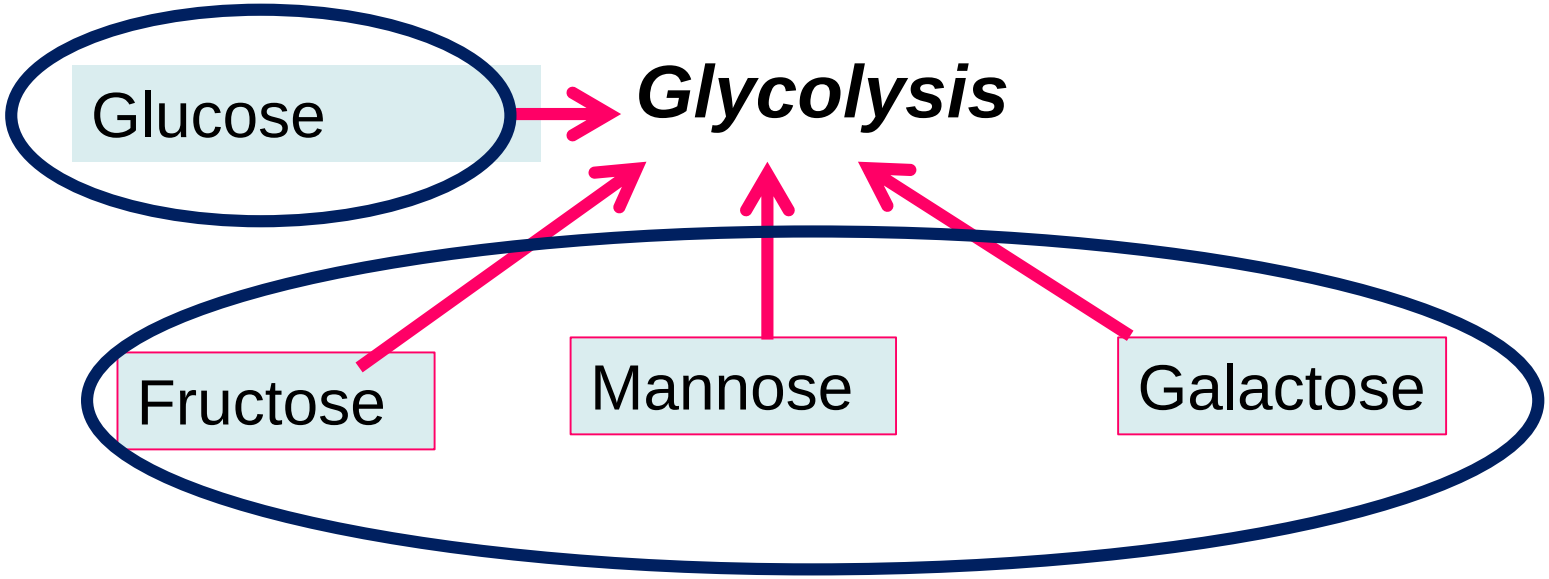
Glucose→ 2 lactate +2H+ ΔG°= -196 kJ/mol

Glucose → 2 CO_2 +2 ethanol ΔG°= -235 kJ/mol

1 glucose molecule → 2 ATP molecules — Fermentation

1 glucose molecule → 32 ATP molecules — Oxidative phosphorilation

Other monosaccharides in the glycolytic pathway (Hexoses)

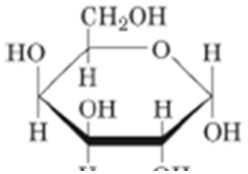


Hexoses other than glucose can undergo glycolysis after conversion to a phosphorylated derivative.

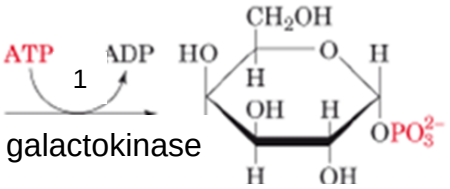
Galactose

D-Galactose + ATP→ Galactose 1-phosphate + ADP

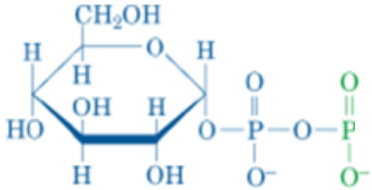
Liver



galactose



Galactose 1-phosphate



UDP-glucose

Uridine

2.Uridyl transferase transfers UMP from UDP-glucose to form UDP-galactose e glucose 1-phosphate

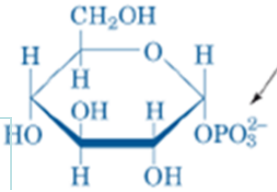
D-Galactose, a hydrolysis product of the of lactose passes in the blood from the intestine to the liver,

2

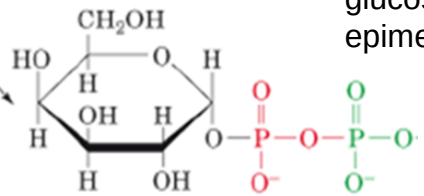
Galactose 1-phosphate uridyl transferase

3

UDP-glucose 4 epimerase



Glucose 1-phosphate



UDP-Galactose

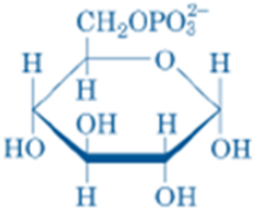
Uridine

1. phosphorylated at C-1, by galactokinase enzyme

3. UDP-galactose is converted to UDP-glucose by UDP-glucose 4 epimerase

4

phosphoglucomutase



Glucose 6-phosphate

Glycolysis

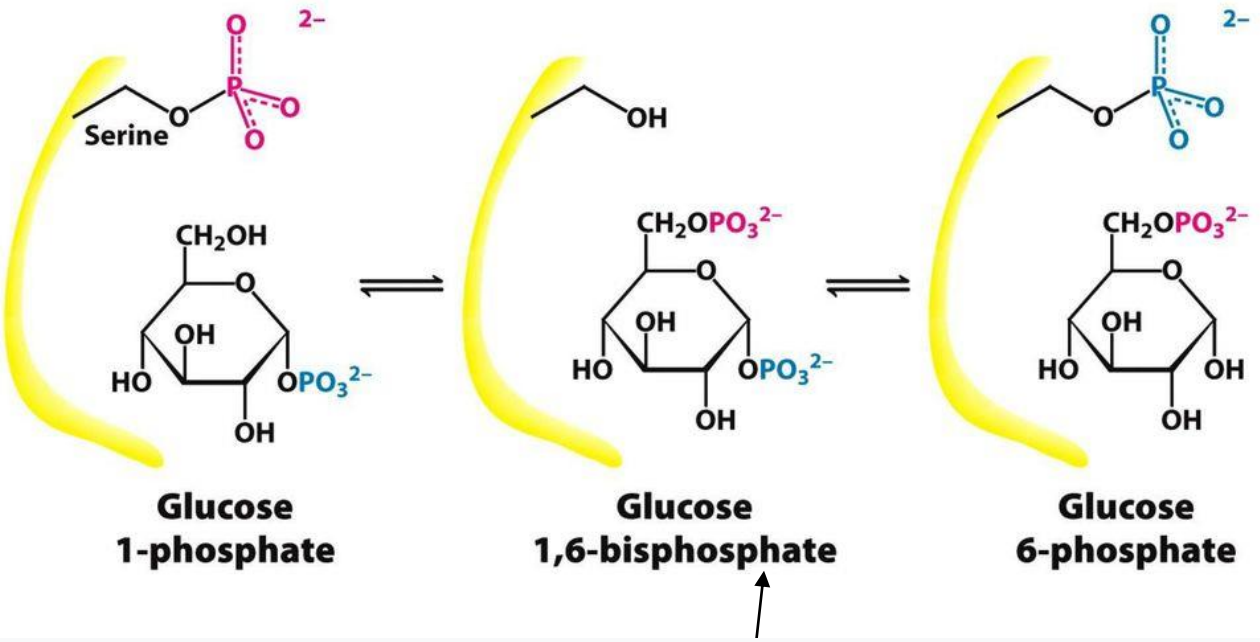
can be directed towards glycolysis

4. **Glucose 1-phosphate is converted into glucose 6-phosphate by phosphoglucomutase**

Galactokinase is Mg2+ dependent and require ATP

Phosphoglucomutase

The mechanism involves a phosphorylated serine.

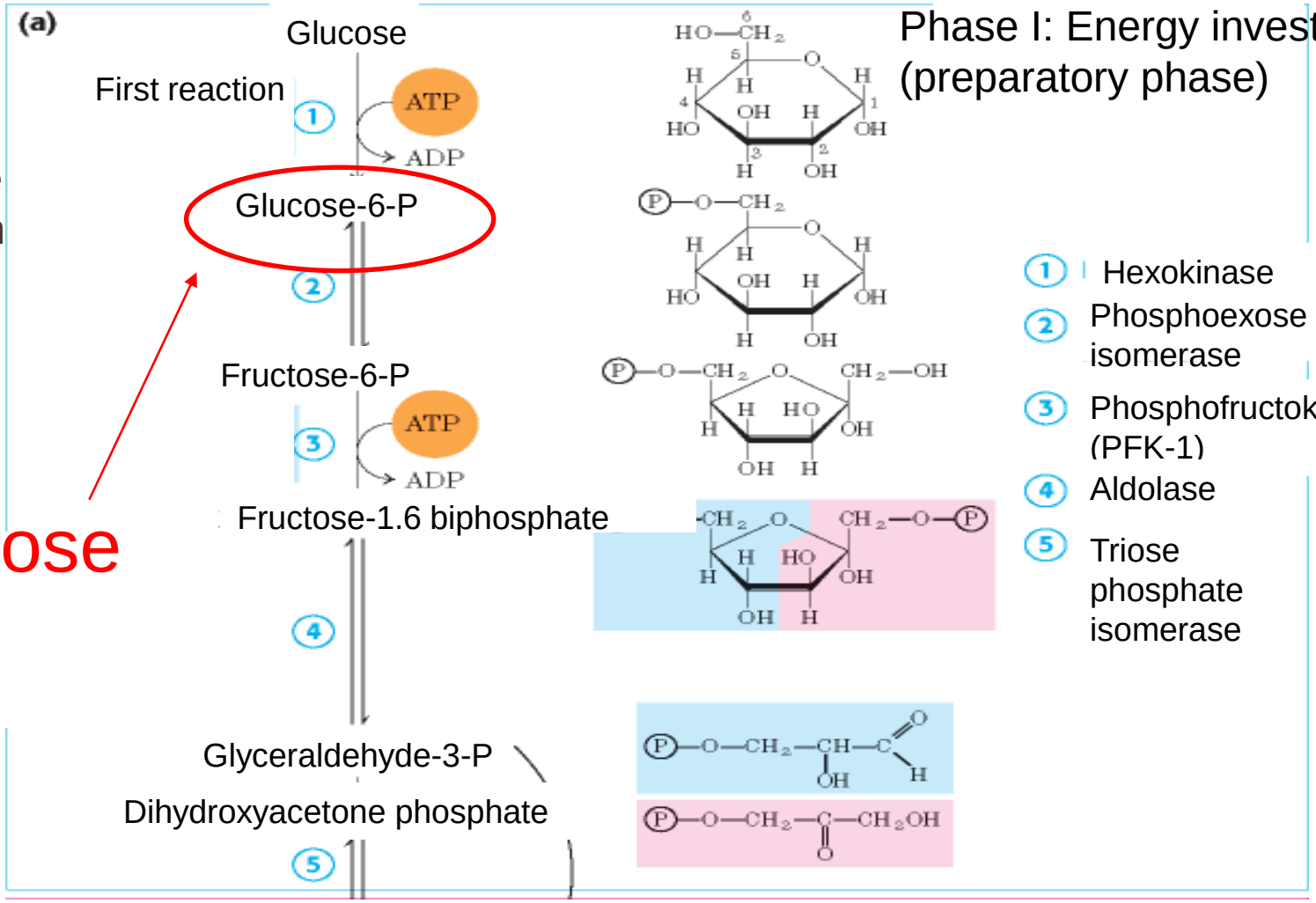


A bisphosphorylated intermediate is formed and is converted to glucose 6 phosphate and serine is rephosphorylated

“Suicide inhibitors”, such as diisopropylfluorophosphate (DFP) irreversibly inhibit the enzyme, through the acylation of the serine essential for catalysis.

First phase requires an investment of 2 ATP molecules

Galactose



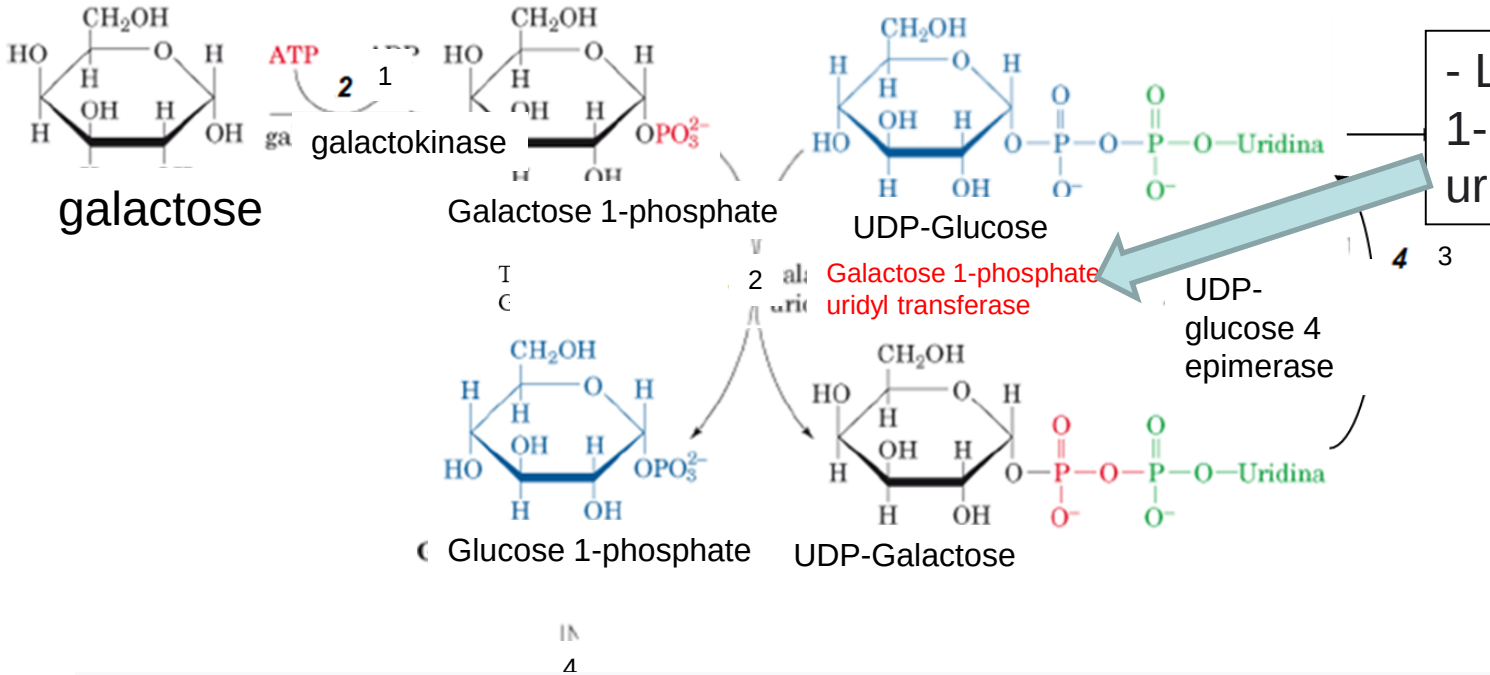
Phase I: Energy investment (preparatory phase)

1. Hexokinase
2. Phosphoexose isomerase
3. Phosphofructokinase 1 (PFK-1)
4. Aldolase
5. Triose phosphate isomerase

Galactosemia

Liver

Galactosemia is a rare genetic metabolic disorders (autosomal recessive)



- Lack galactose 1-phosphate uridyltransferase

- Galactose 1-phosphate accumulates in the liver
- The first symptom is the presence of jaundice.
-

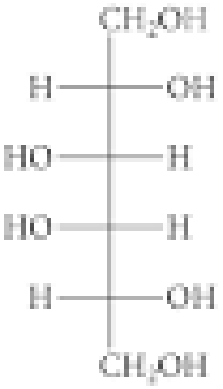
Galactosemia

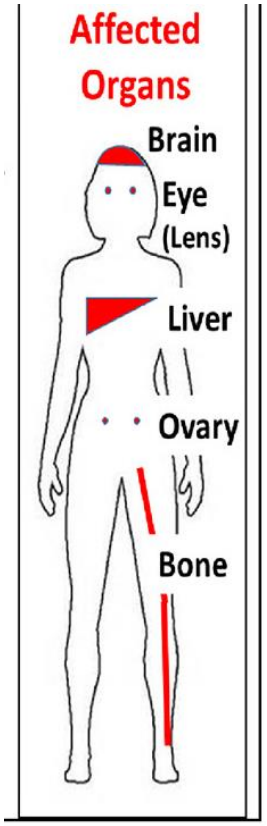
-Other central nervous system effects are also observed

(growth retardation and mental delay)

- The increase in the blood concentration of galactose causes an increase in the glucide in the lens, where it is reduced to galactitol

- Galactitol causes cataracts in the lens





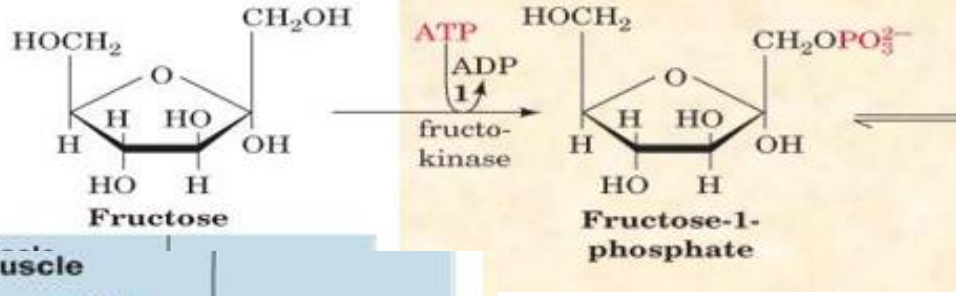
A diet low in galactose and lactose is important to mitigate the most serious effects

- It is possible to detect galactose1-phosphate uridyltransferase in the red blood cells of the umbilical cord at birth

Fructose

D-Fructose, present in free form in many fruits.
Formed by hydrolysis of sucrose in the small intestine of vertebrates
Phosphorylated by hexokinase

D-Fructose + ATP $\xrightarrow{Mg^{2+}}$ fructose 6-phosphate + ADP



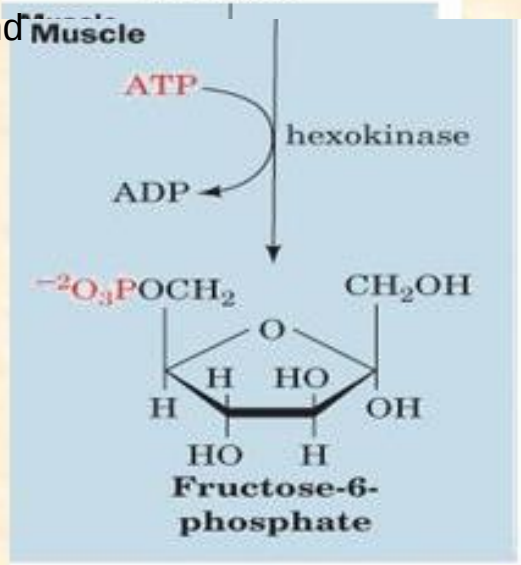
LIVER

1. Fructokinase catalyzes the phosphorylation in C-1

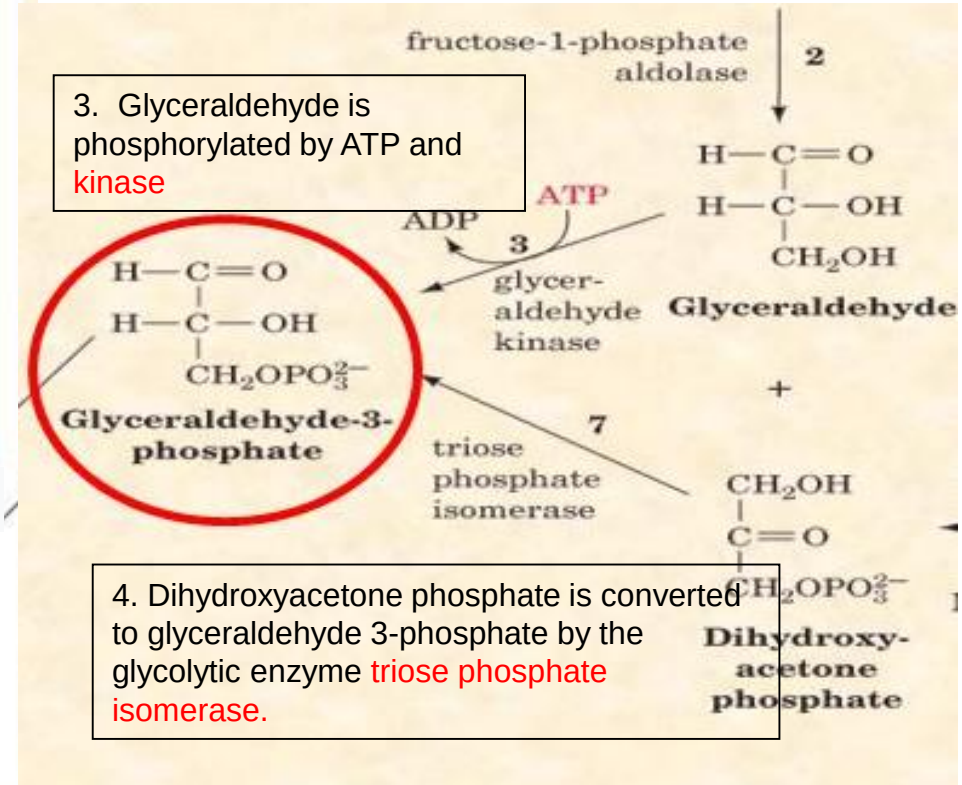
Fructose + ATP → fructose 1-phosphate + ADP

2. The fructose 1-phosphate is cleaved by fructose 1-phosphate aldolase:

Kidney and Muscle



can be directed towards glycolysis



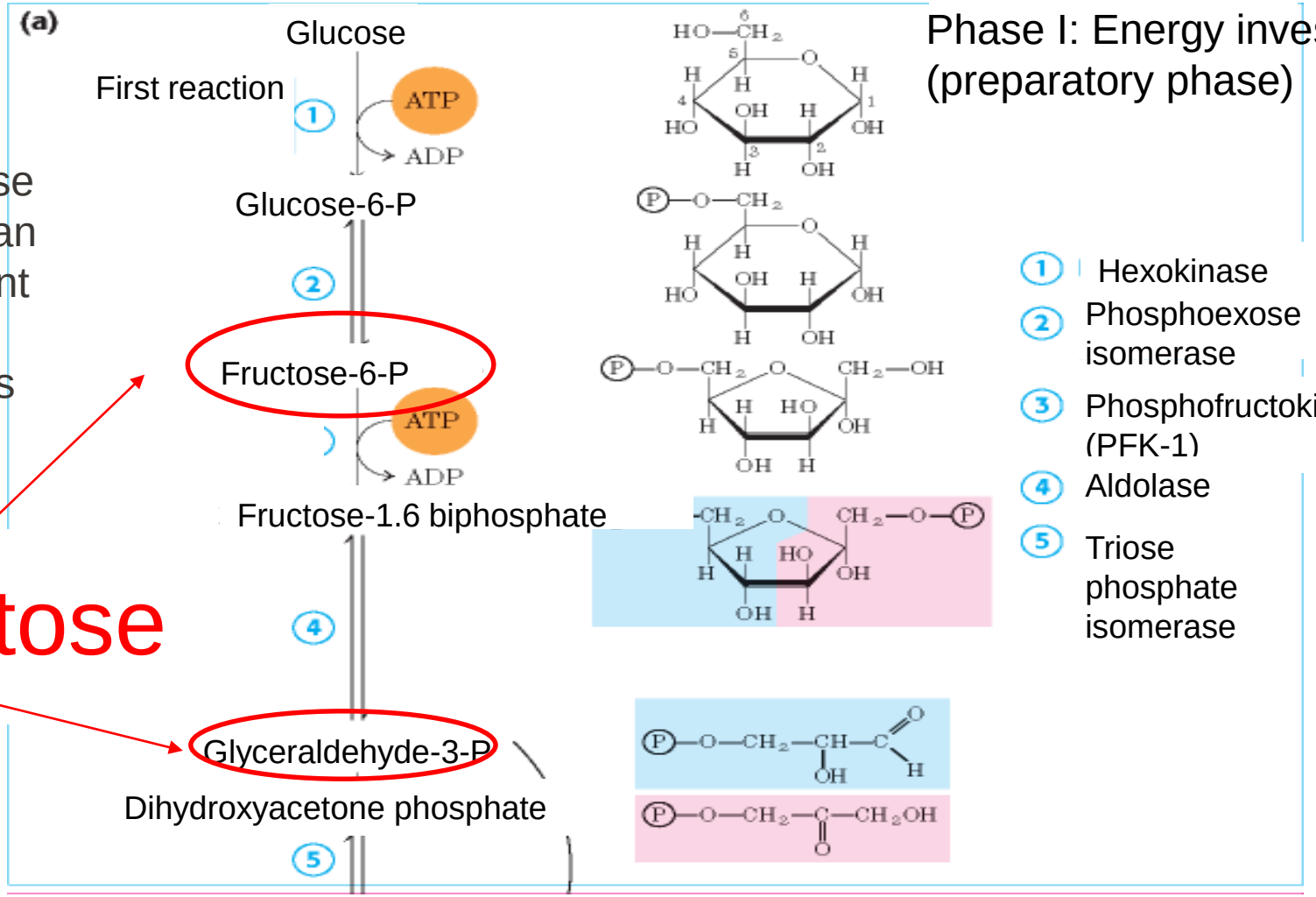
3. Glyceraldehyde is phosphorylated by ATP and kinase

4. Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate by the glycolytic enzyme triose phosphate isomerase.

Phase I: Energy investment (preparatory phase)

First phase requires an investment of 2 ATP molecules

Fructose



1. Hexokinase
2. Phosphoexose isomerase
3. Phosphofructokinase 1 (PFK-1)
4. Aldolase
5. Triose phosphate isomerase

Fructose intolerance

Hereditary Fructose Intolerance (HFI),

Fructose intolerance is due to the aldolase deficiency **(autosomal recessive)**

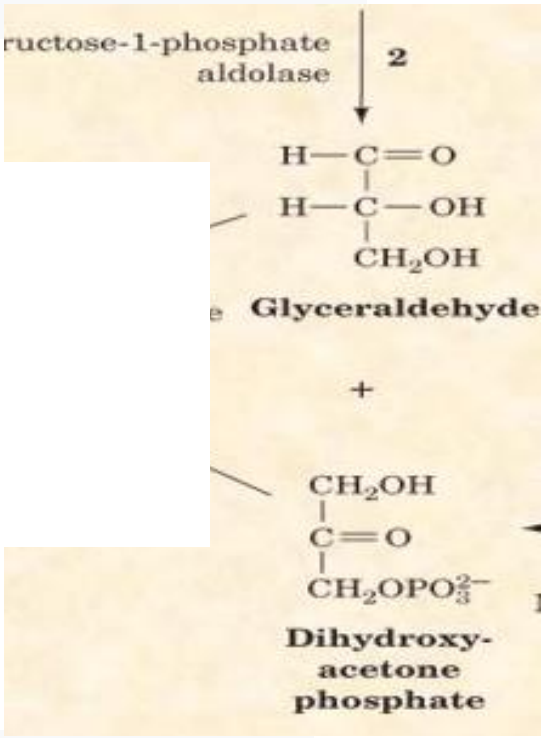
Fructose + ATP → fructose 1-phosphate + ADP

The disease is caused by a mutation in a gene called AldoB

Fructose1-phosphate accumulates until the phosphate stores in the liver are depleted.

The concentration of ATP decreases and causes liver damage

Furthermore, the increase in the concentration of fructose 1-phosphate inhibits:



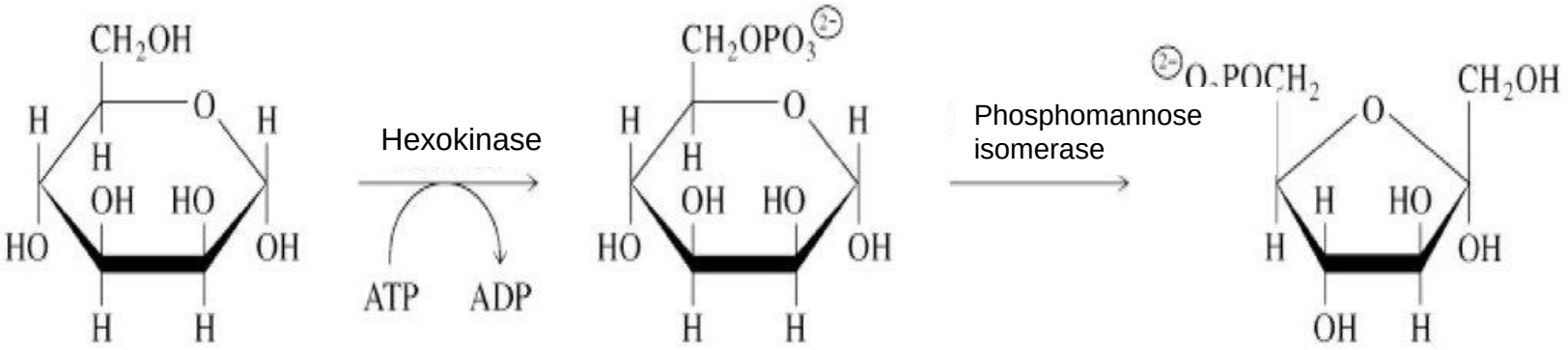
glycogen phosphorylase (an enzyme necessary for the breakdown of glycogen to glucose)

fructose 6-phosphatase (an enzyme necessary in gluconeogenesis (glucose synthesis),

Mannose

LIVER

D-Mannose can be phosphorylated at C-6 by hexokinase:



Mannose

Mannose 6-P

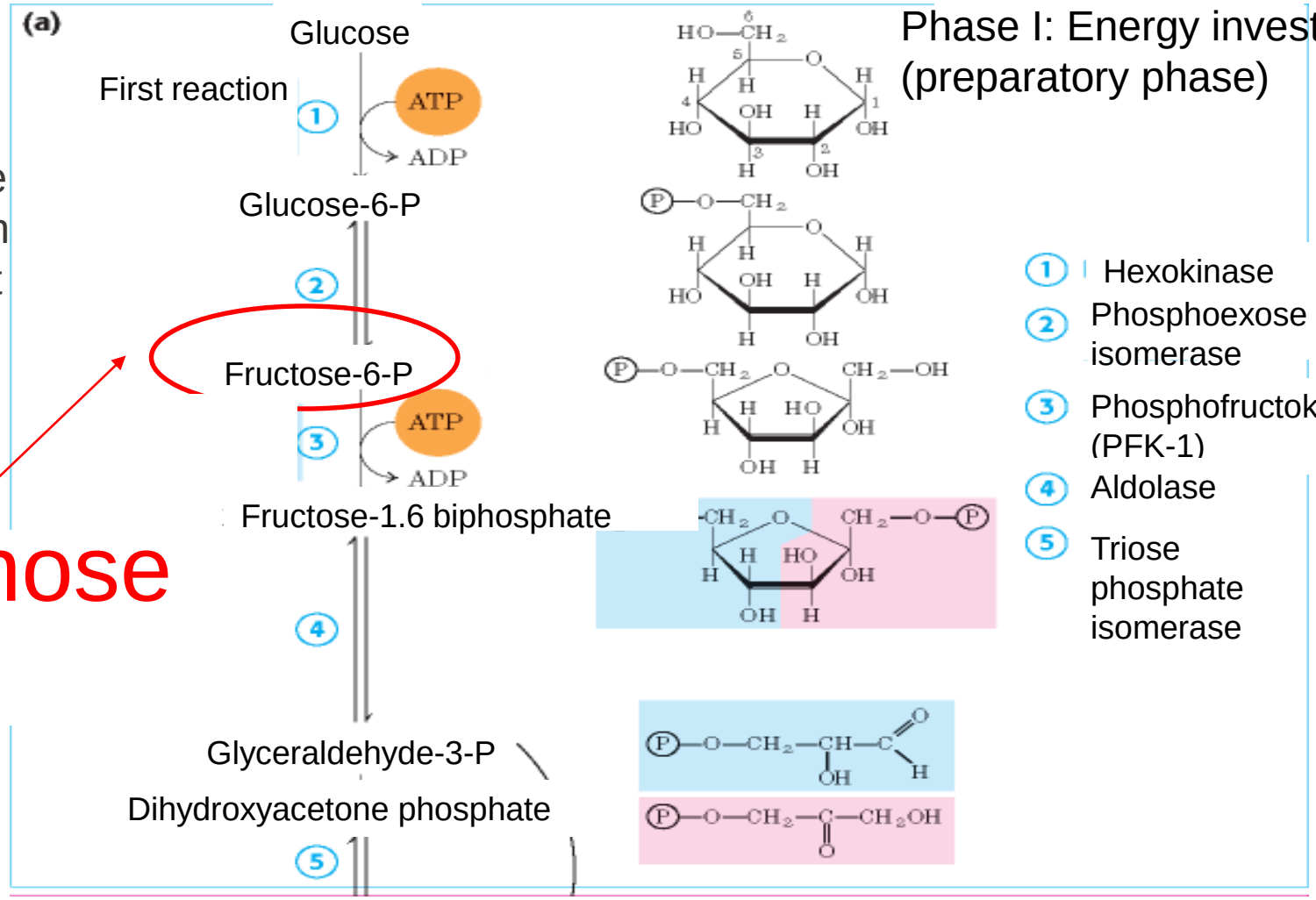
Fructose 6 P

Mannose 6-phosphate is isomerized by phosphomannose isomerase to yield fructose 6-phosphate, an intermediate of glycolysis.

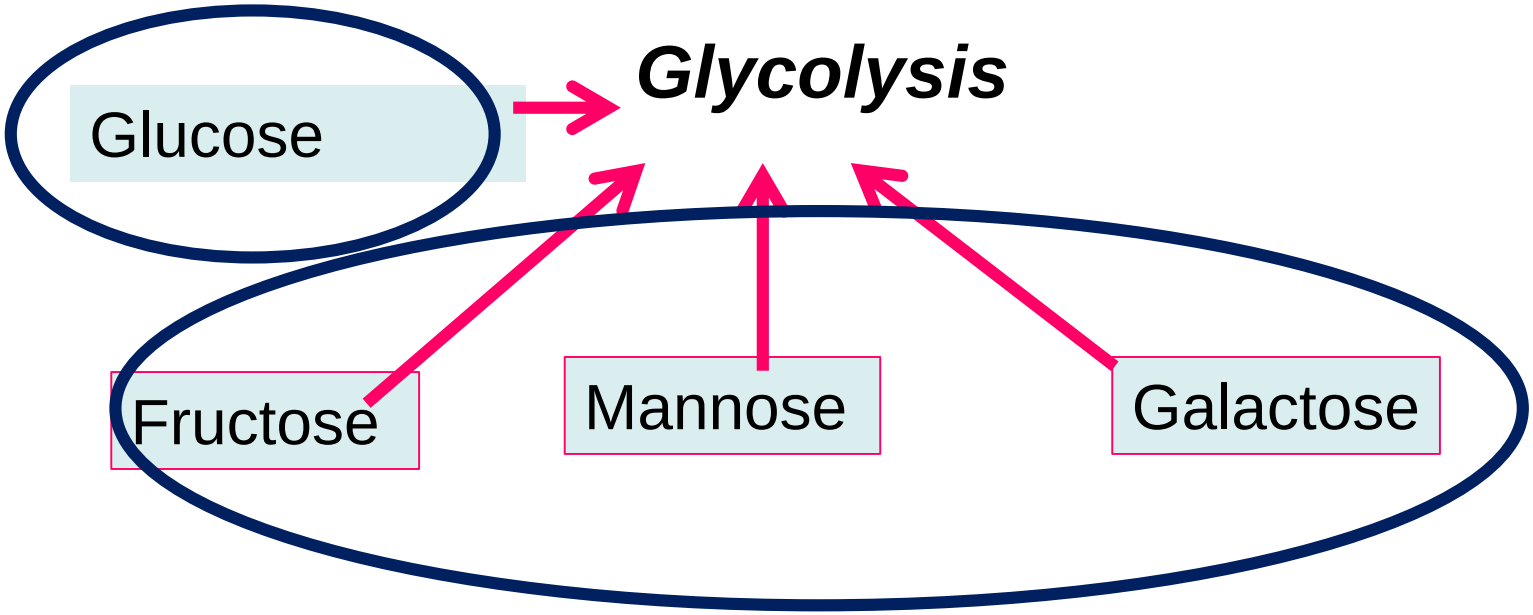
Phase I: Energy investment (preparatory phase)

First phase requires an investment of 2 ATP molecules

Mannose

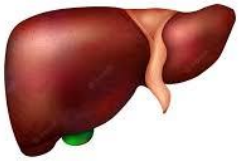


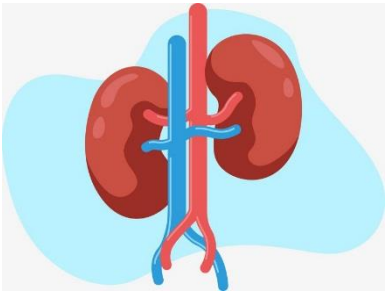
1. Hexokinase
2. Phosphoexose isomerase
3. Phosphofructokinase 1 (PFK-1)
4. Aldolase
5. Triose phosphate isomerase

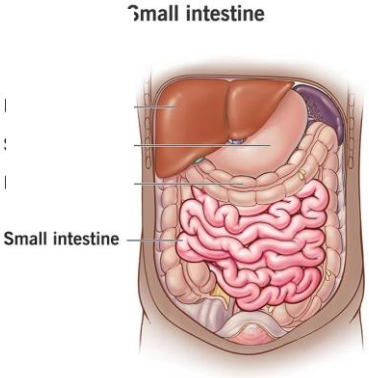

Glucose
Glycolysis
Fructose
Mannose
Galactose

Gluconeogenesis

Occurs in the liver and to a lesser extent kidneys and small intestine

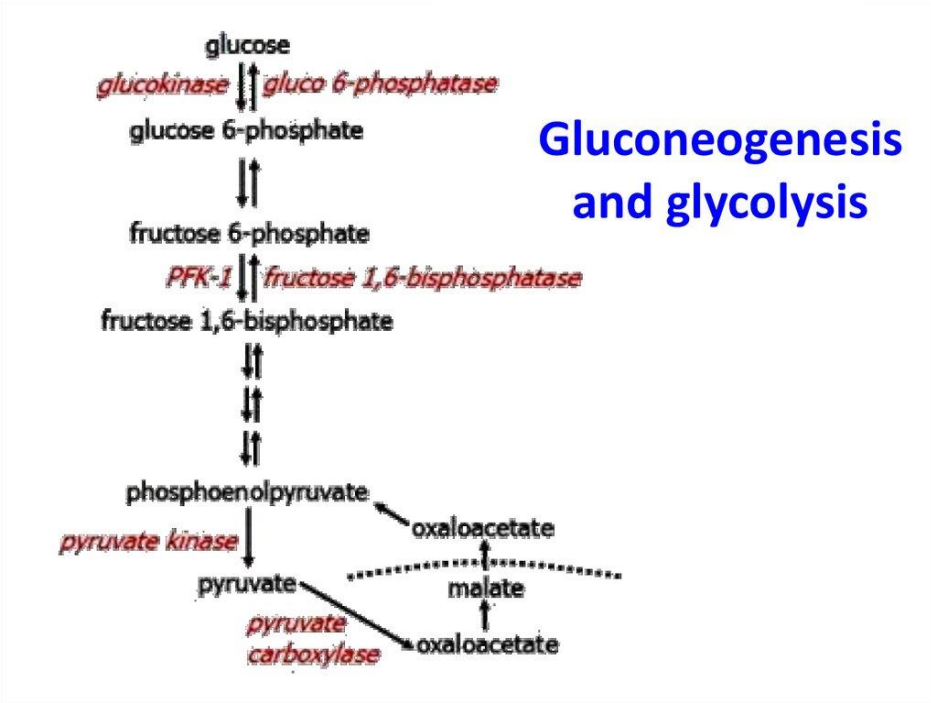




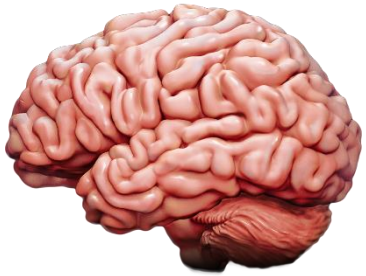


The liver and kidney can synthesize glucose from lactate, pyruvate, and amino acids (non saccharides precursor)

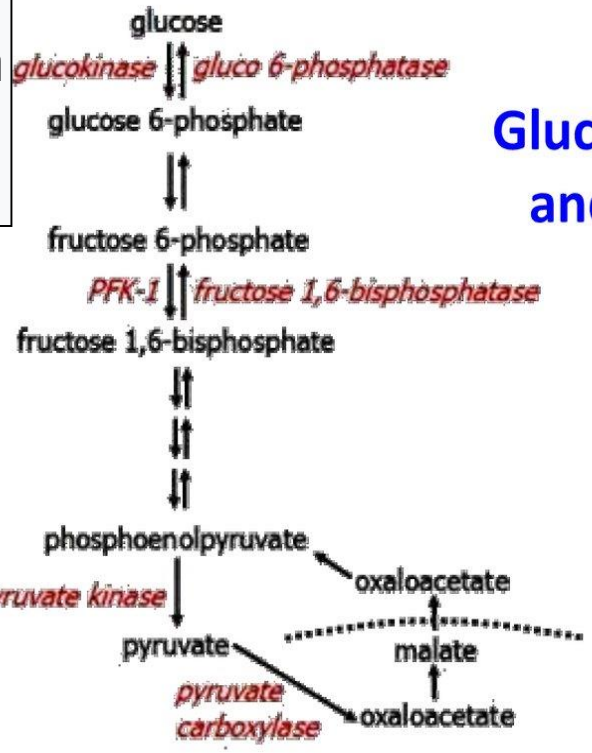
Under fasting conditions, gluconeogenesis supplies almost all of the body's glucose.



Glucose is required for metabolism in tissues such as the brain



The muscle, during exercise, converts glucose into pyruvate and lactate which go to the liver to be converted back to glucose.



Gluconeogenesis and glycolysis

Muscle and brain use a large part of the newly synthesized glucose.

- Gluconeogenesis is mostly the reverse of glycolysis

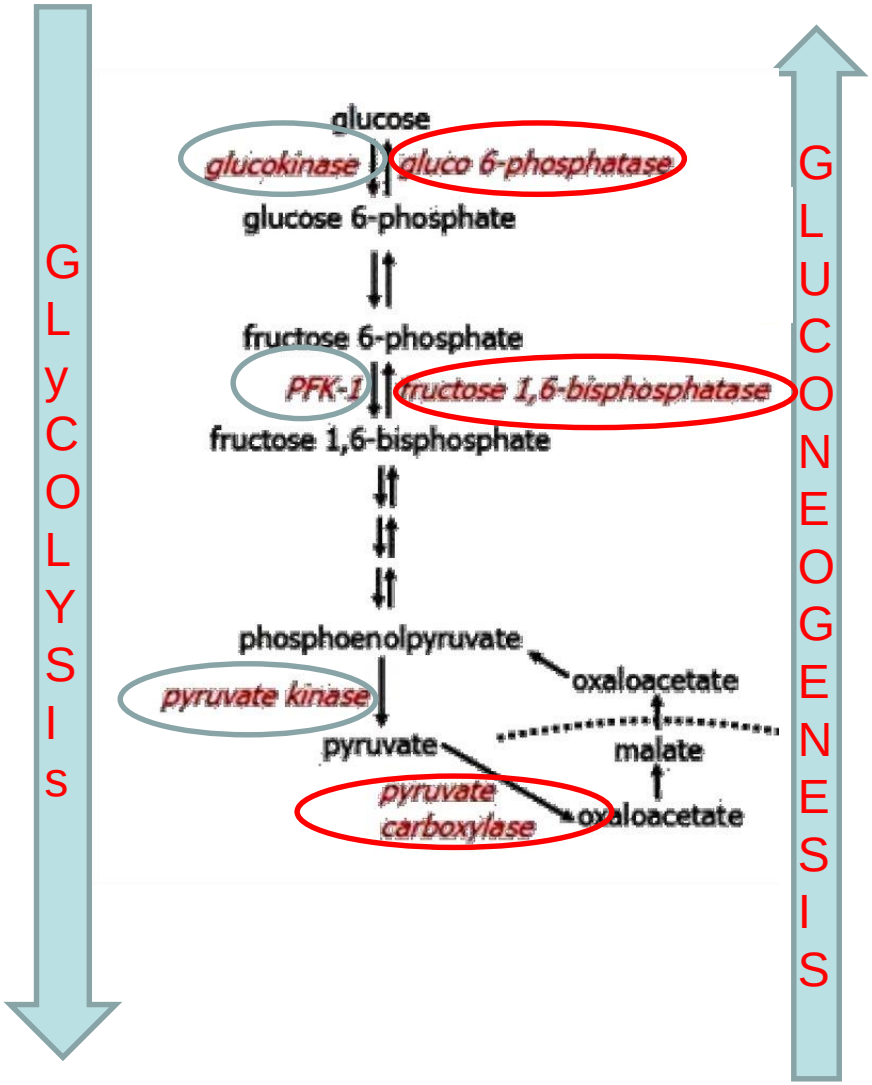
Glycolysis and gluconeogenesis differ in three step.

Three step are irreversible in the glycolysis which have exergonic ΔG

- hexokinase
- phosphofructokinase 1 (PFK1)
- pyruvate kinase

They are catalyzed by different enzymes in biosynthesis

-Glucose 6-phosphatase
- Fructose 1,6 biphosphatase
- Pyruvate carboxylase



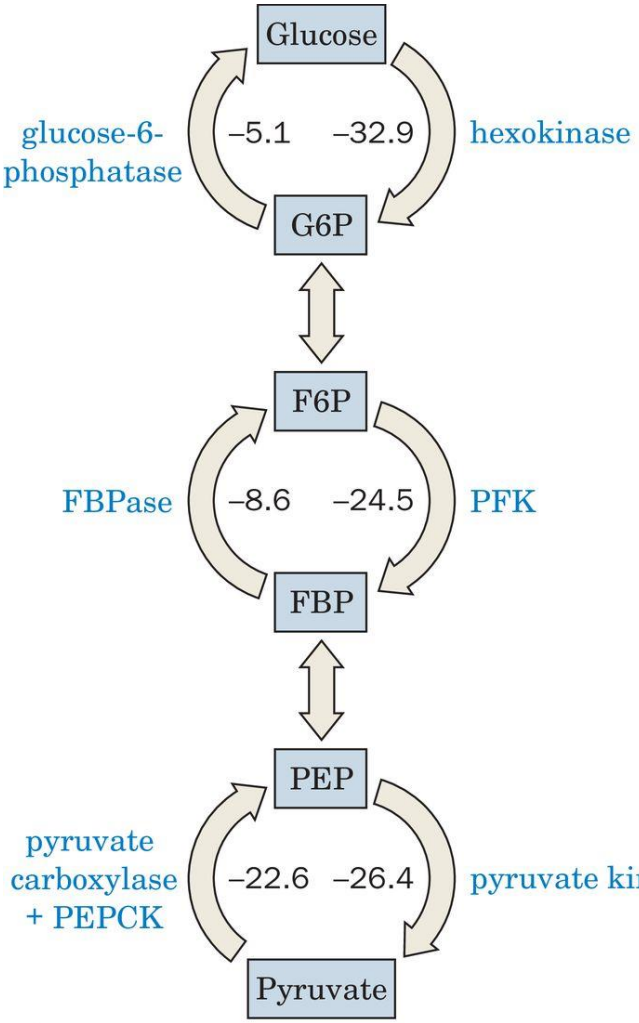
Gluconeogenesis

- Byosynthesis of 1 glucose molecule requires:
- 4 ATP
- 2 NADH
- 2 GTP

- 2 pyruvate + 2 NADH + 4 ATP + 2GTP + 6 H_2O + 2 H^+ -----→
- Glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 Pi

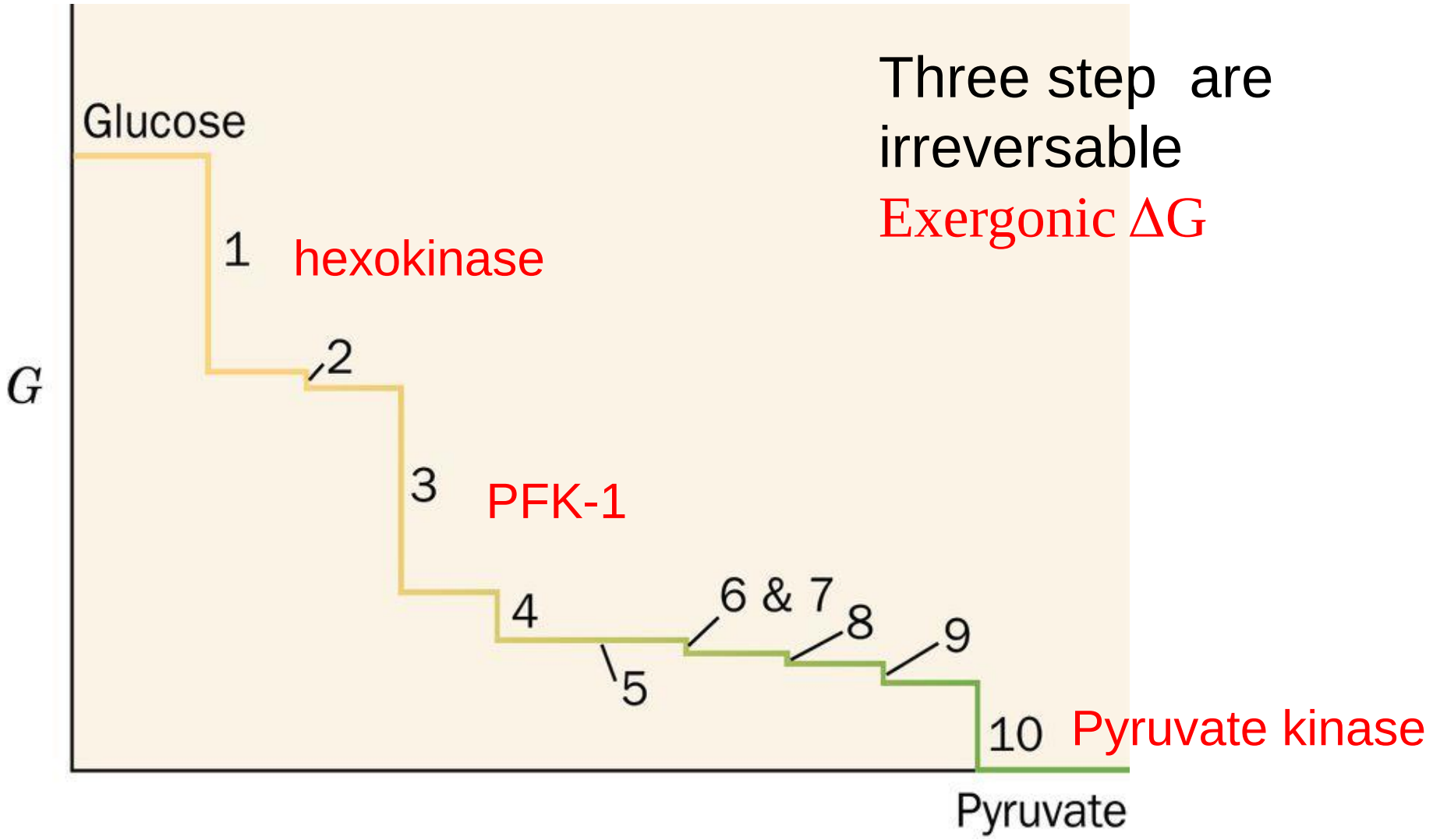
- Glycolysis and gluconeogenesis are reciprocally regulated by allosteric effects, phosphorylation, and changes in enzyme synthesis rates.

The enzymes involved are regulated so that both pathways are EXERGONIC by SUBSTRATE CYCLES



ΔG's obtained from Newsholme, E.A. and Leech, A.R., Biochemistry for the Medical Sciences, p. 448, Wiley (1983).

Diagram of Free Energy Changes



Three step are irreversable

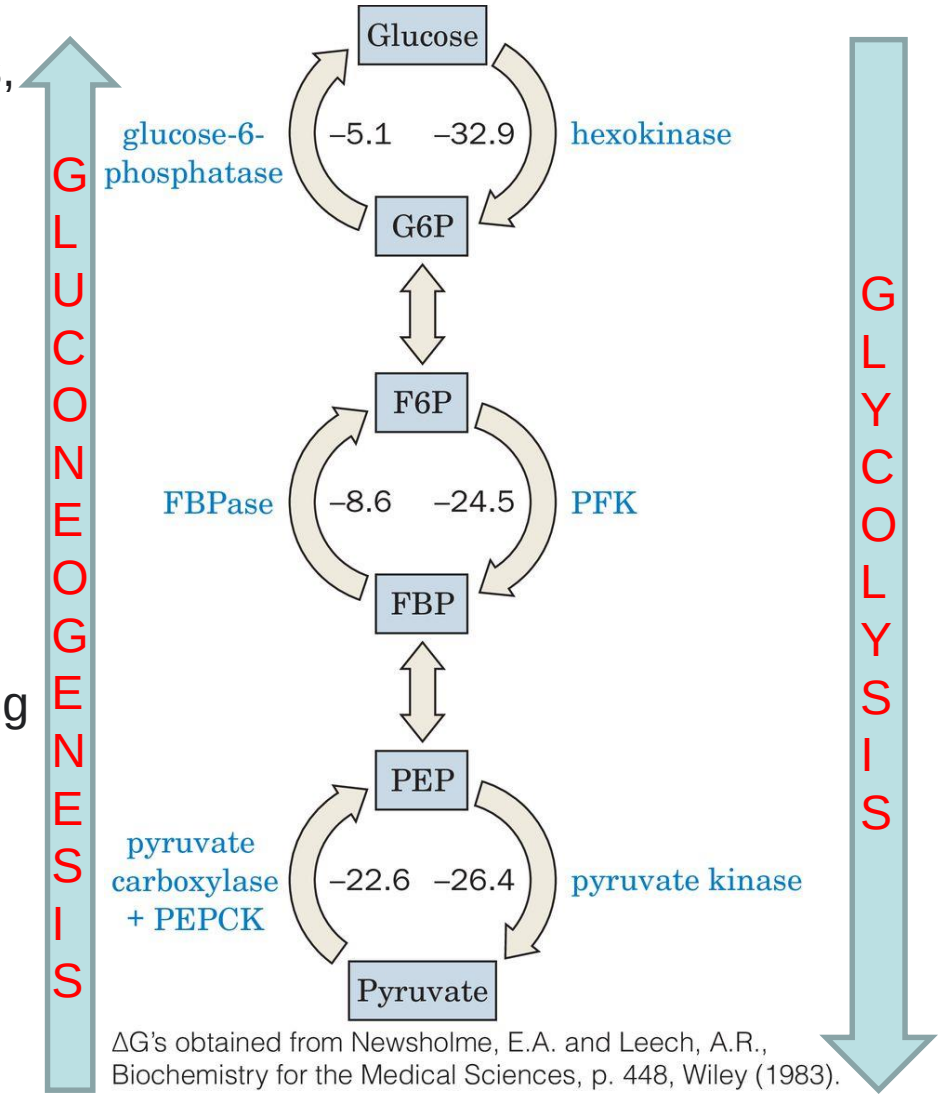
Exergonic ΔG

Substrate Cycles in Glucose Metabolism

In the absence of regulatory mechanisms, in the three points of deviation of the gluconeogenesis

the simultaneous functioning of both pathways could give rise to a so-called "FUTILE CYCLE"

(reaction that disperses energy resulting from the hydrolysis of ATP without carrying out any net metabolic work)



ΔG's obtained from Newsholme, E.A. and Leech, A.R., Biochemistry for the Medical Sciences, p. 448, Wiley (1983).

Glycolysis and gluconeogenesis are mutually regulated.

The regulation is at the level of the deviation points

GLYCOLYSIS REGULATION:

The three checkpoints of glycolysis are the reactions catalyzed by:

- Hexokinase
- Phosphofructokinase-1 (PFK-1)
- Pyruvate kinase

GLUCONEOGENESIS REGULATION:

The three checkpoints of gluconeogenesis are the reactions catalyzed by:

-Glucose 6-phosphatase
- Fructose 1,6 biphosphatase
- Pyruvate carboxylase

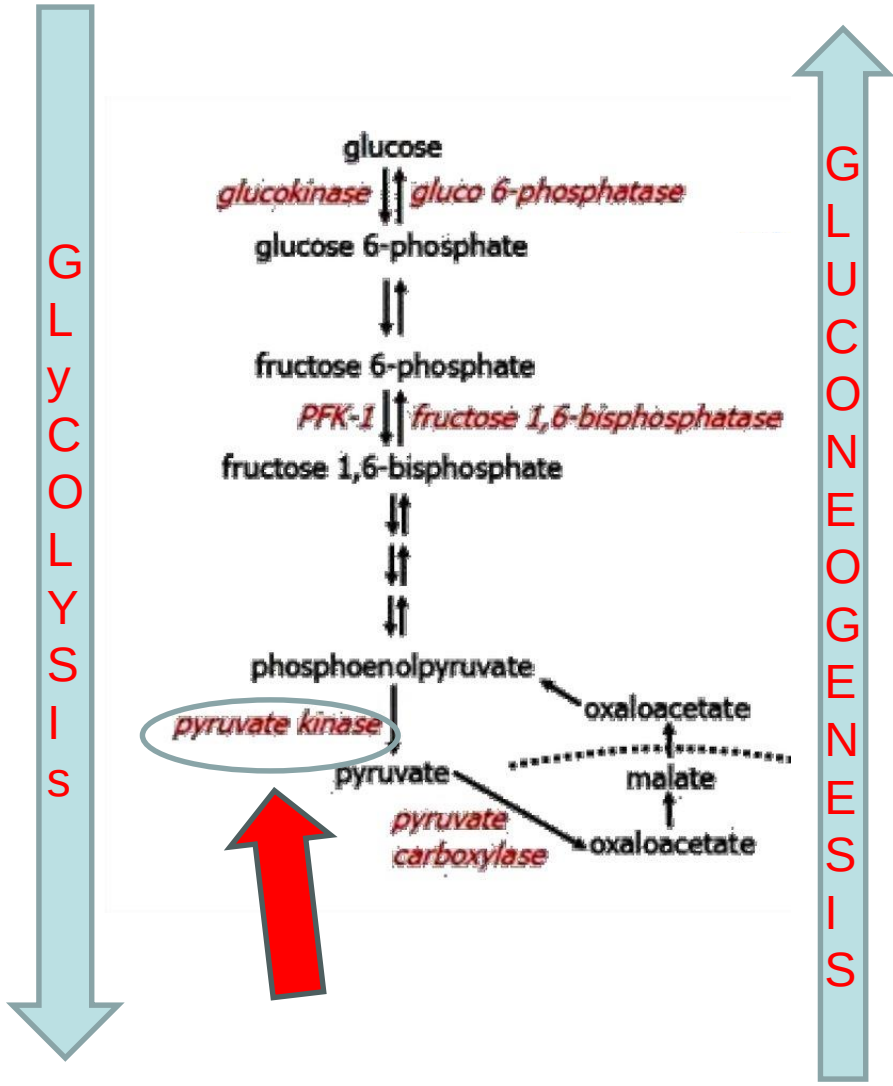
Gluconeogenesi *vs* Glycolysis

Pyruvate --→ PEP
requires two reactions

mitochondria 1 cytosol 2

PYRUVATE →OXALOACETATE→ PEP

First step to regulation



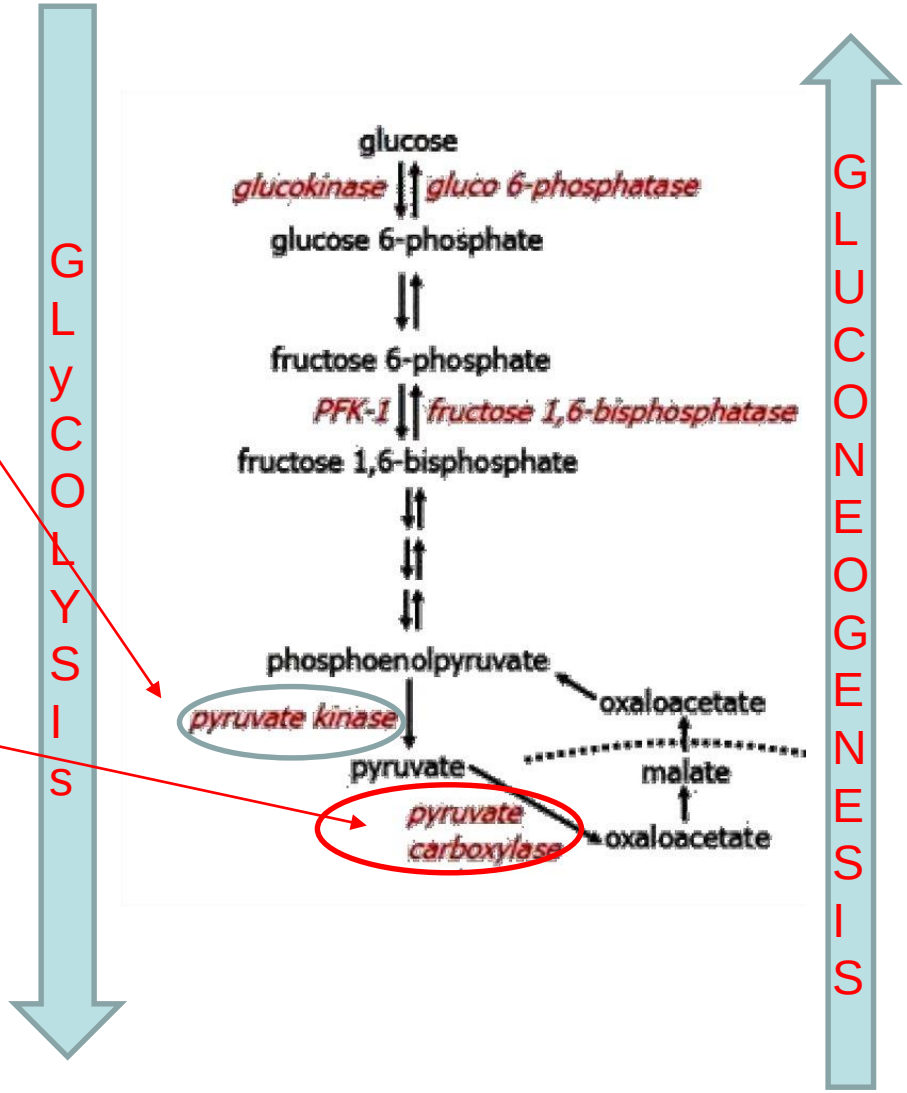
Glycolysis

- pyruvate kinase
- phosphofructokinase 1
- hexokinase

Gluconeogenesis

-pyruvate carboxylase

- **Fructose 1,6 biphosphatase**
- **Glucose 6-phosphatase**

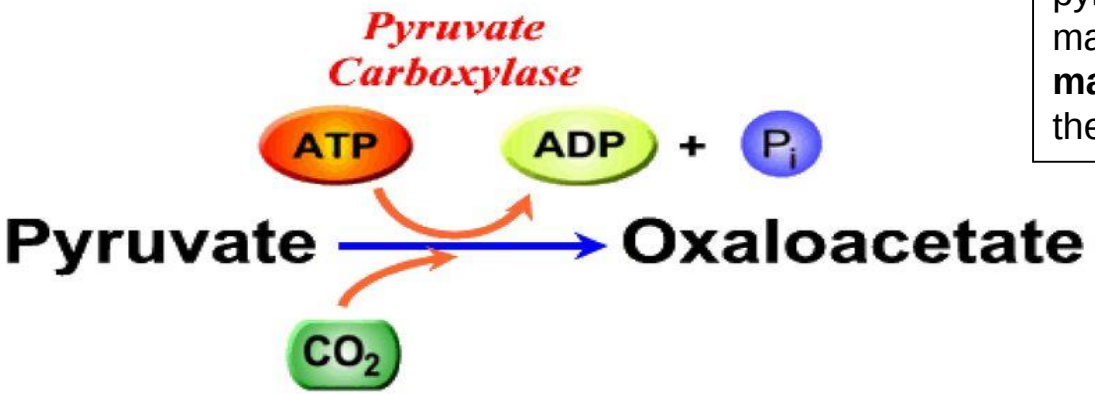


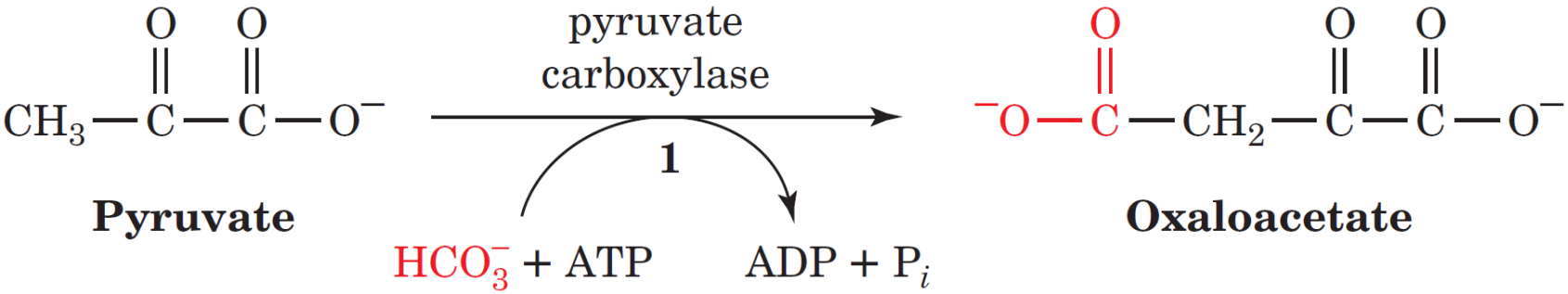
Pyruvate carboxylase

liver and kidney but absent in muscle

- **ATP, biotin, Mn^{++} and CO_2 are required.**

Because the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial **malate dehydrogenase**, at the expense of NADH:

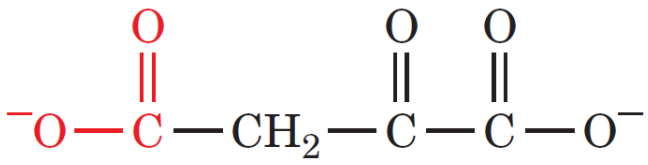




mitochondria cytosol

PYRUVATE $\xrightarrow{1}$ OXALOACETATE $\xrightarrow{2}$ PEP

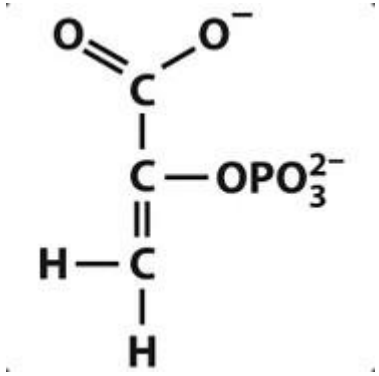
Cytosol



Oxaloacetate

GTP GDP

PEPCK



PEP

Phosphoenolpyruvate carboxokinase

Requires GTP as donor to phosphoric group

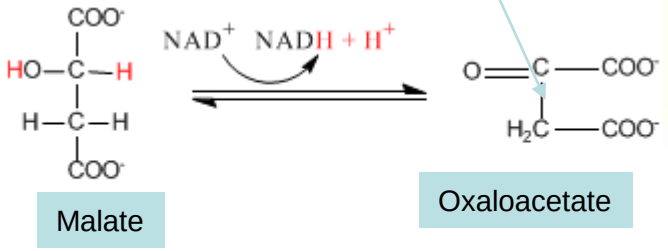
Phosphoenolpyruvate carboxokinase of bacteria used ATP as phosphate donor while in animals used GTP

Gluconeogenesis require a shift of metabolites between cytosol and mitochondrion

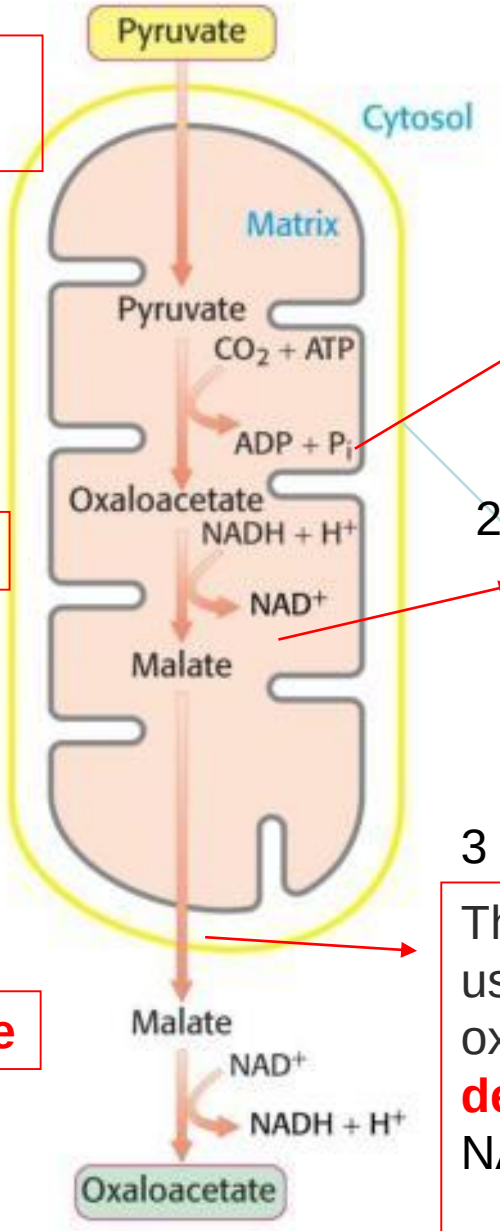
Pyruvate carboxylase
Is only mitochondrial Enzyme

Pyruvate carboxylase

Malate dehydrogenase



Malate dehydrogenase



1

Oxaloacetate does not have a carryer into mitochondrial membrane

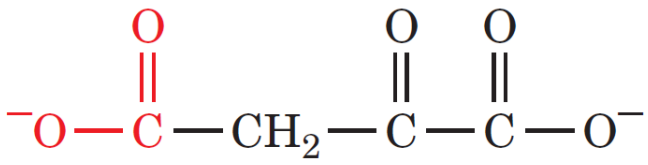
2

Oxaloacetate is reduced to **malate** by the mitochondrial **malate dehydrogenase.**
NADH + H+ -> NAD+

3

The **malate** enters the cytosol using an exchanger protein and is oxidized by the cytosolic **malate dehydrogenase** to oxaloacetate.
NAD+ +H+ -> NADH

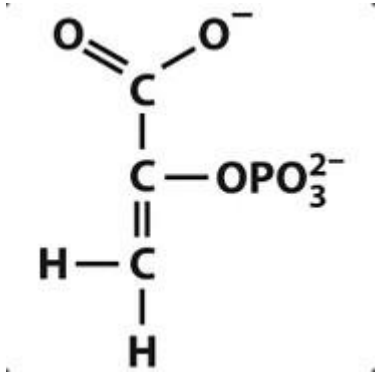
Cytosol



Oxaloacetate

GTP GDP

PEPCK



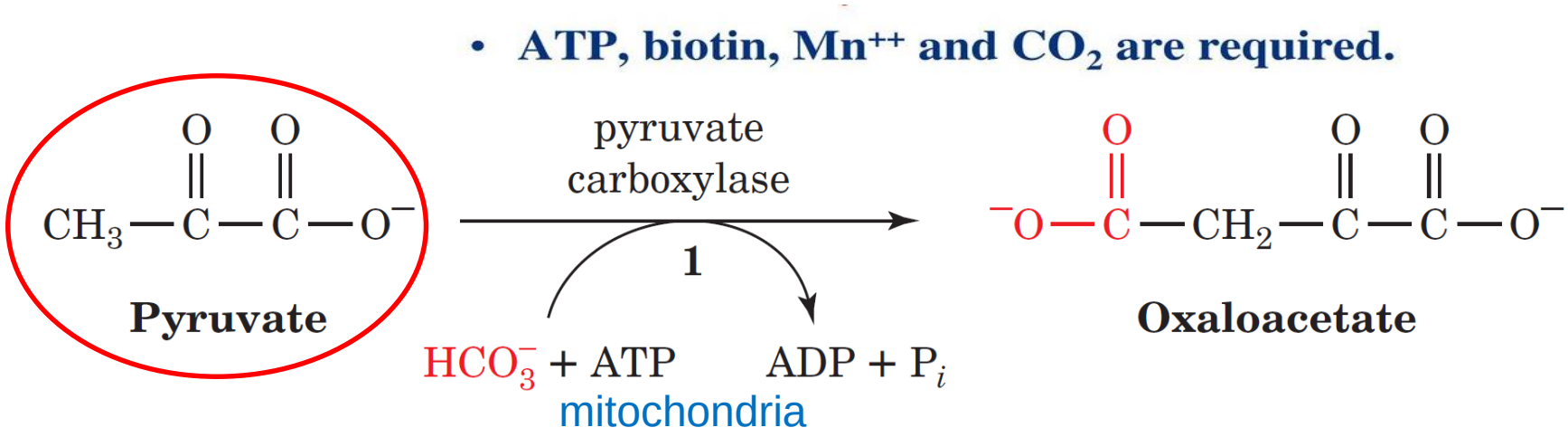
PEP

Phosphoenolpyruvate carboxokinase

Requires GTP as donor to phosphoric group

Phosphoenolpyruvate carboxokinase of bacteria used ATP as phosphate donor while in animals used GTP

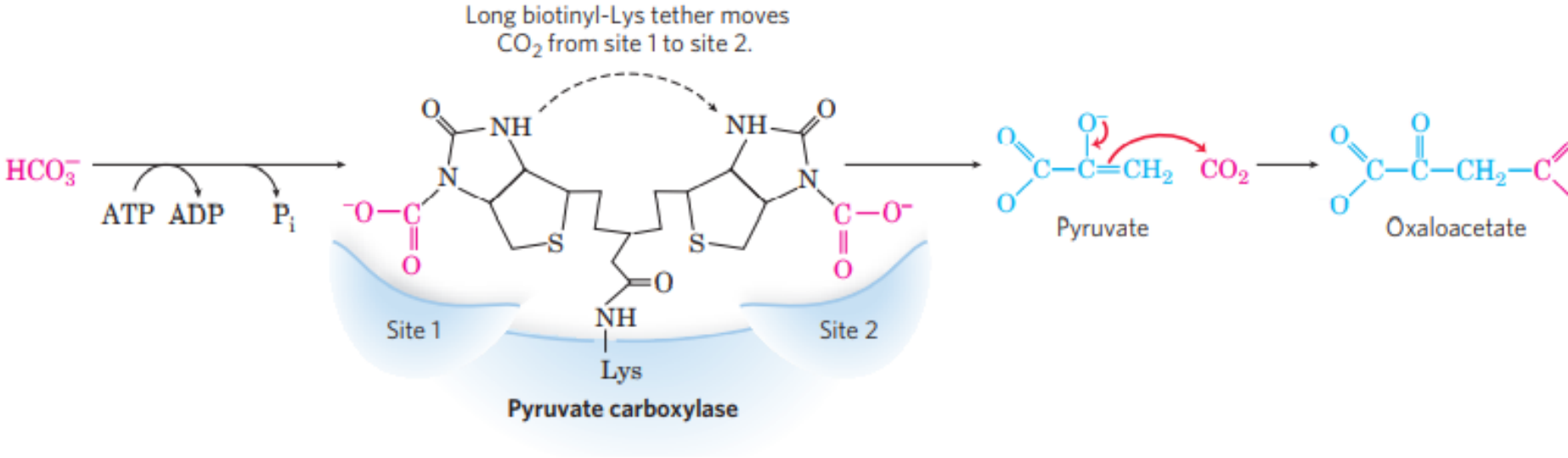
Pyruvate Carboxylase Reaction



Carboxylation involving pyruvate, bicarbonate ion, biotin and ATP

Mechanism

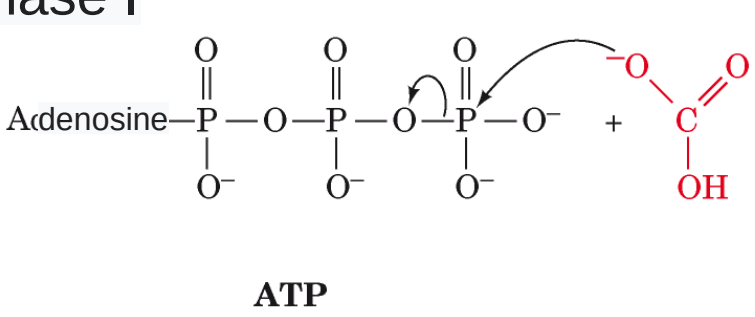
Biotin is covalently attached to the enzyme through an amide linkage to the ε´-amino group of a Lys residue, forming a biotinyl-enzyme.

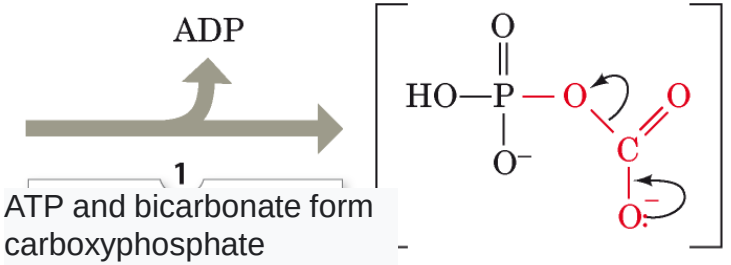


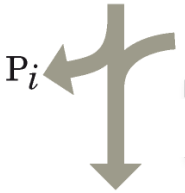
Mechanism

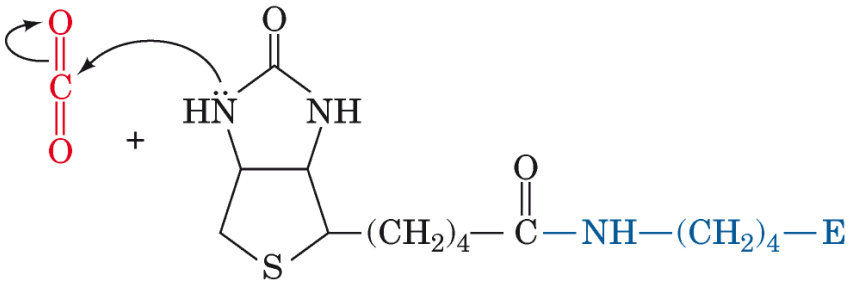
The reaction occurs in two phases, which occur at two different sites in the enzyme

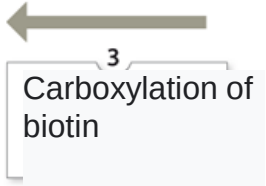
Phase I

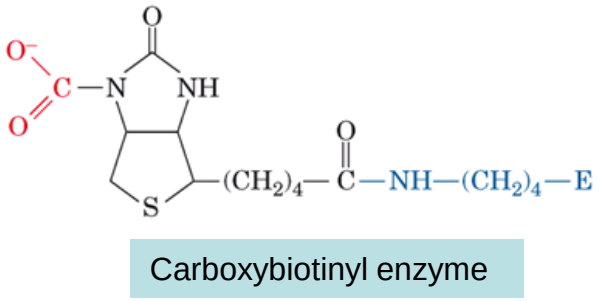




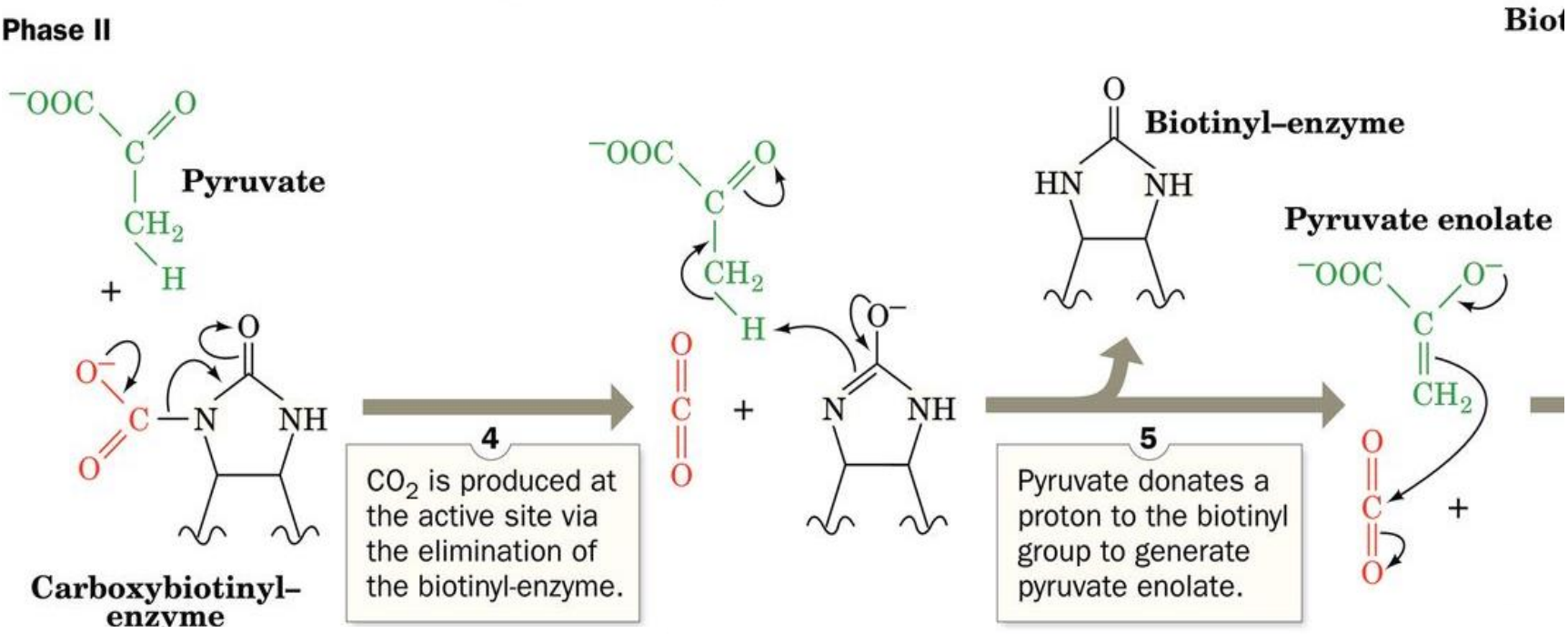






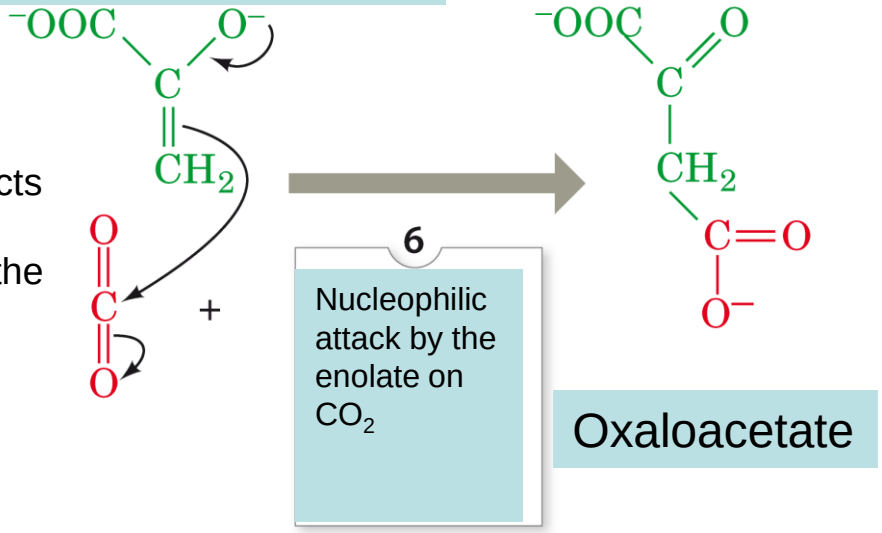


At catalytic site 1, bicarbonate ion is converted to CO2 at the expense of ATP. Then CO2 reacts with biotin, forming carboxybiotinyl-enzyme



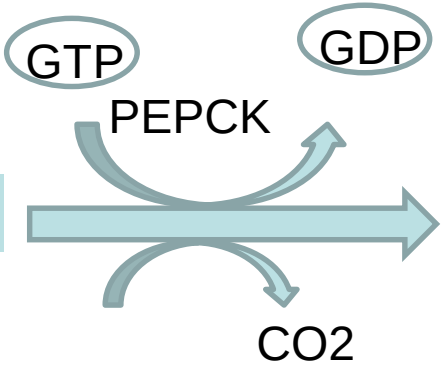
. The long arm composed of biotin and the Lys side chain to which it is attached then carry the CO_2 of carboxybiotinyl-enzyme

where CO2 is released and reacts with the pyruvate, forming oxaloacetate and regenerating the biotinyl-enzyme.



Phosphoenolpyruvate carboxokinase

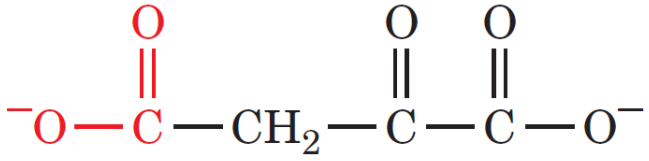
CYTOSOL



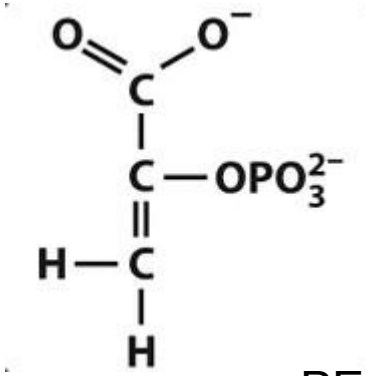
Requires GTP as donor to phosphoric group

Oxaloacetate

PHOSPHOENOLPYRUVATE



Oxaloacetate



PEP

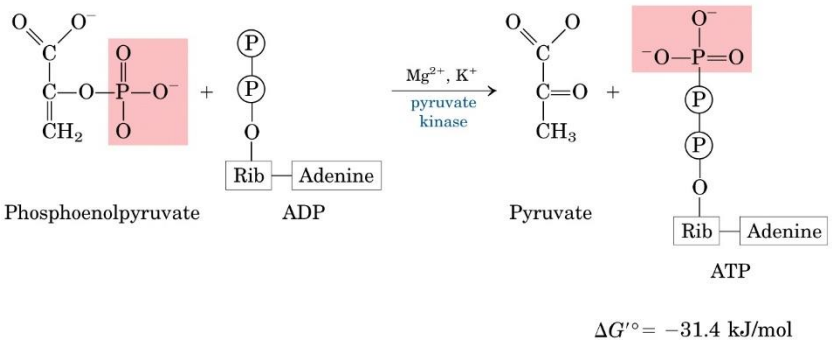
PEPCK Phosphoenolpyruvate carboxokinase

Phosphoenolpyruvate carboxokinase of bacteria used ATP as phosphate donor while in animals used GTP

mitochondria cytosol

PYRUVATE $\xrightarrow{1}$ OXALOACETATE $\xrightarrow{2}$ PEP

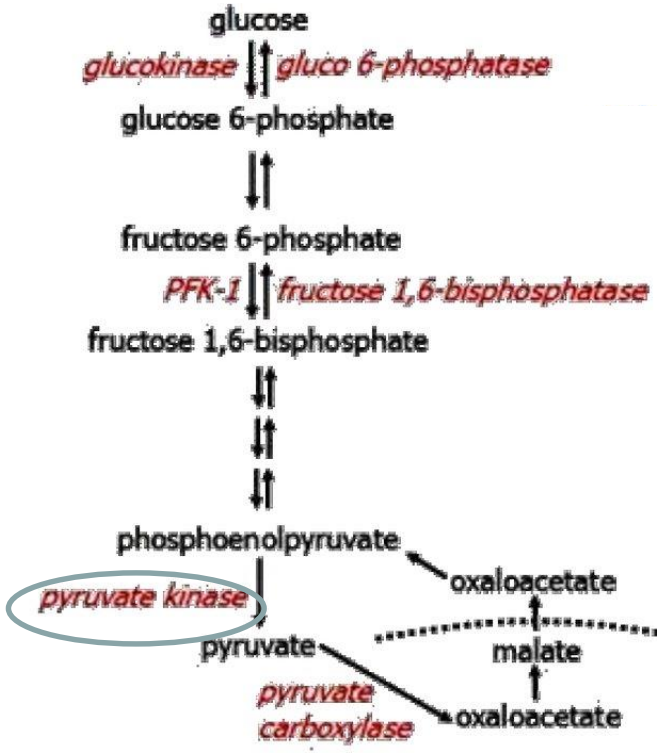
Gluconeogenesi *vs* Glycolysis



Pyruvate kinase

PEP----------→ PYRUVATE

GLYCOLYSIS



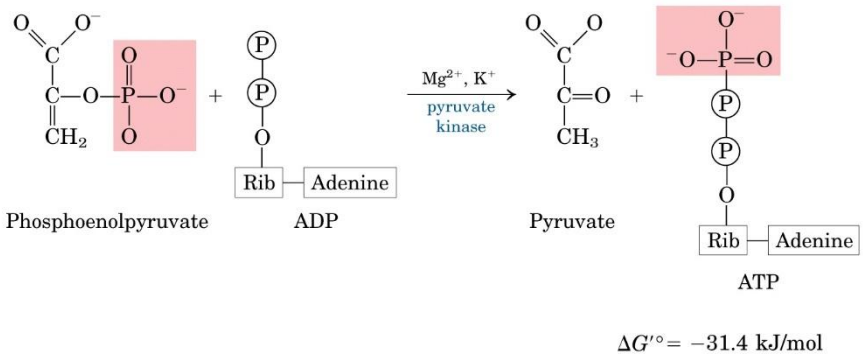
GLUCONEOGENESIS

First step to regulation

Pyruvate kinase regulation (glycolysis)

Requires Mg^{2+} e K^{+}

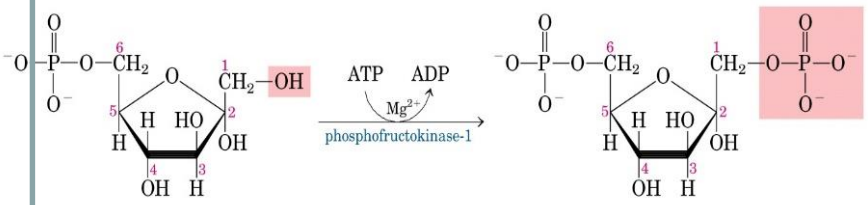
- Allosteric regulation
- Covalently modification
- Hormonal regulation (glucagon)



$\Delta G'^{\circ} = -31.4$ kJ/mol

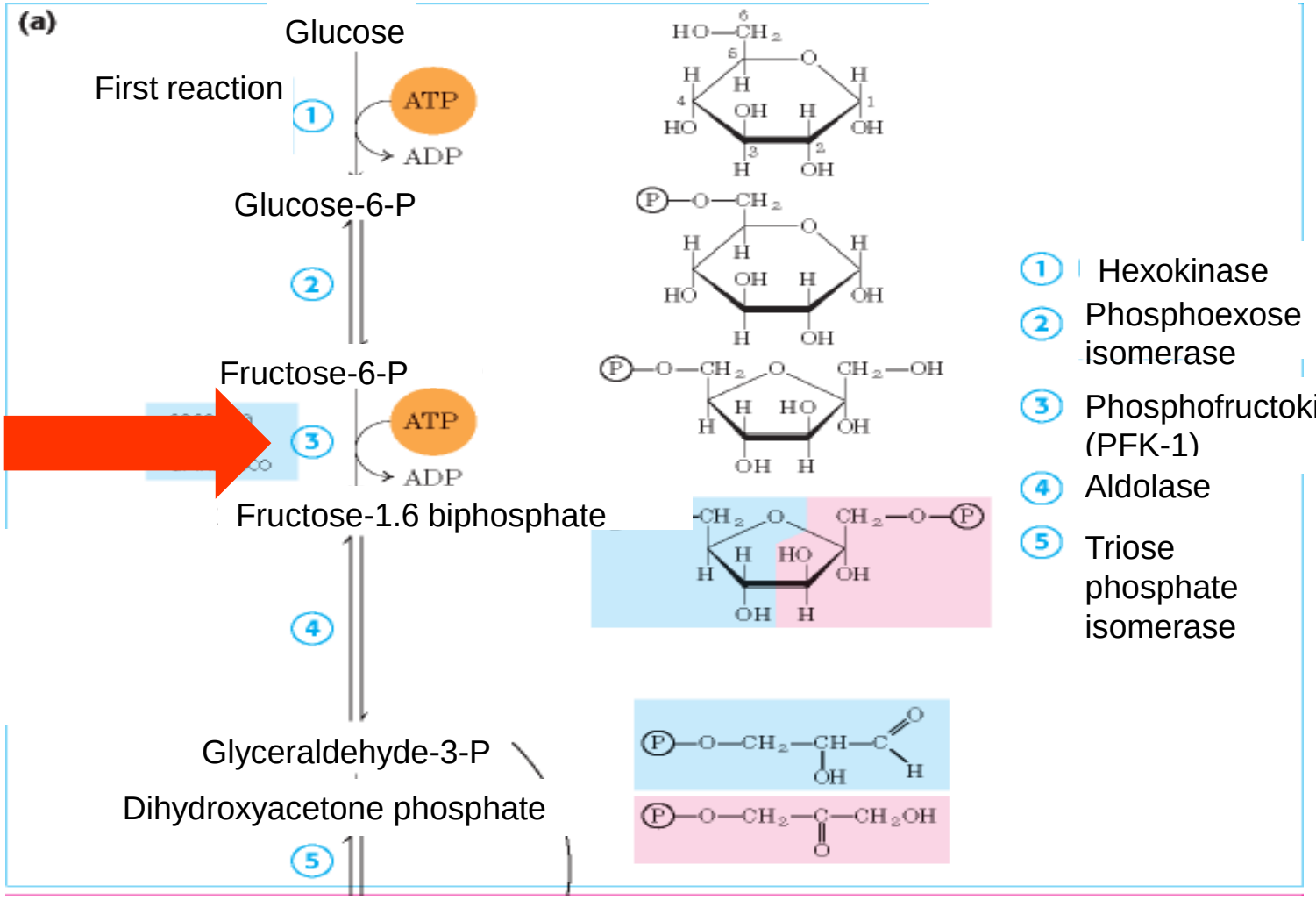
Activator allosteric most important is fructose 1,6-biphosphate (glycolysis intermediate) (3 step)

(3 step of glycolysis)



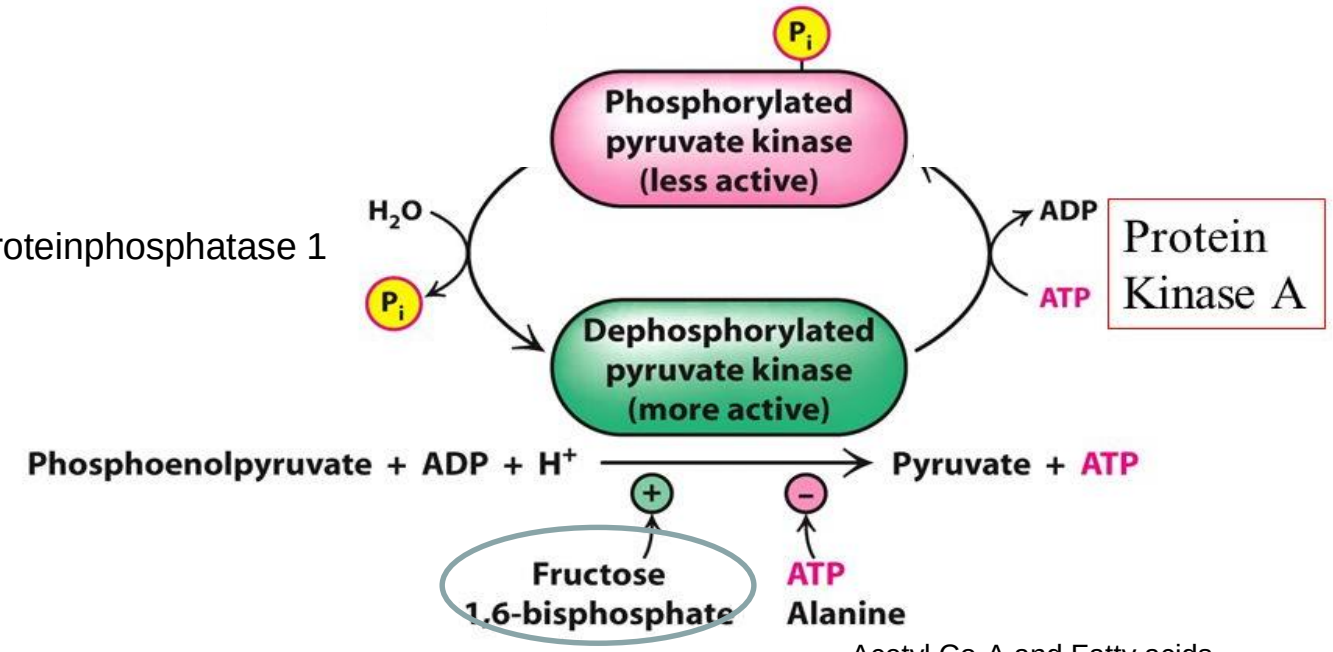
$\Delta G'^{\circ} = -14.2$ kJ/mol

Glycolysis


(a)
Glucose
First reaction
1
ATP
ADP
Glucose-6-P
2
Fructose-6-P
3
ATP
ADP
Fructose-1.6 biphosphate
4
Glyceraldehyde-3-P
Dihydroxyacetone phosphate
5
1 Hexokinase
2 Phosphoexose isomerase
3 Phosphofructokinase 1 (PFK-1)
4 Aldolase
5 Triose phosphate isomerase

Pyruvate kinase regulation (glycolysis)

- Covalently modification



Phosphoproteinphosphatase 1

(PKA)

Acetyl Co-A and Fatty acids

Allosteric regulation

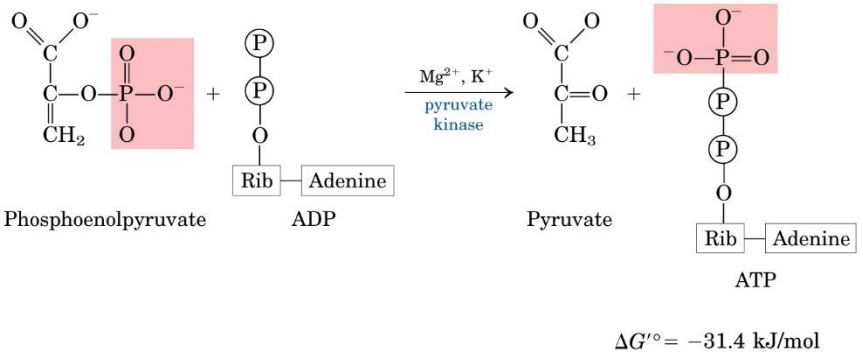
Allosteric inhibition of pyruvate kinase

- ATP
- Acetyl Co-A,
- Long chain fatty acids (signs of abundant availability of energy)
- Alanine (synthetized by pyruvate by 1 step)

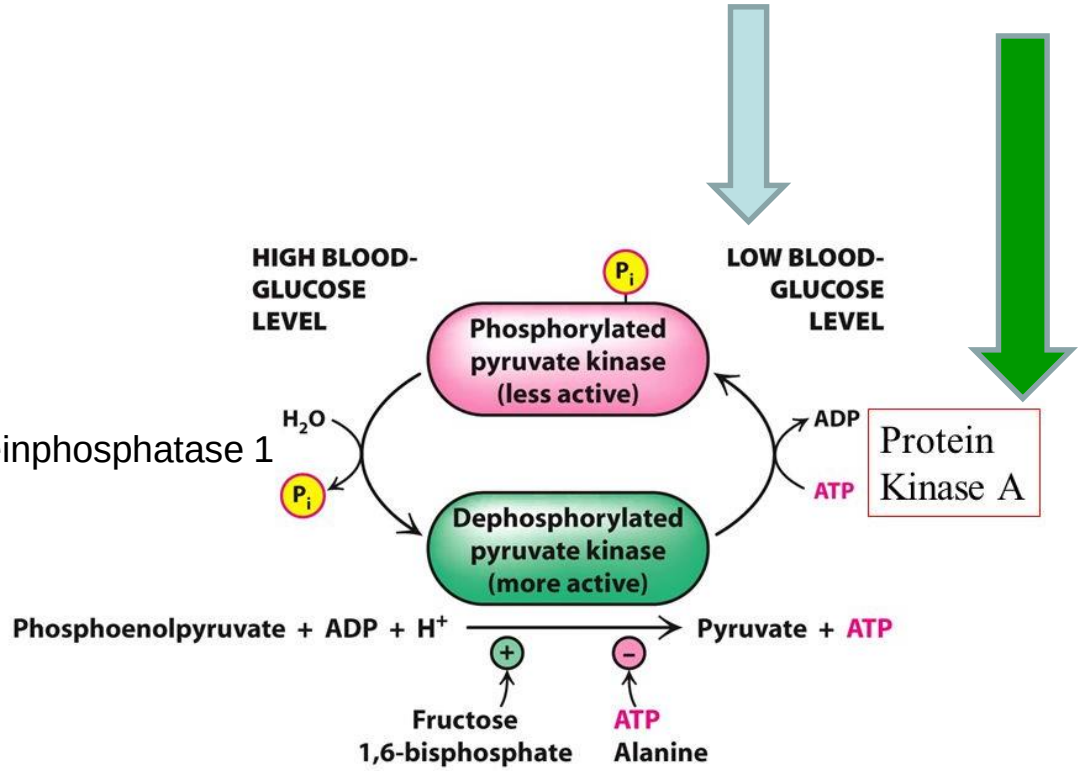
} glycolysis is inhibited

Pyruvate kinase regulation

- Hormonal regulation (glucagon)



When [glucose] decreases --→ glucagon-----→ Protein kinase A (PKA)

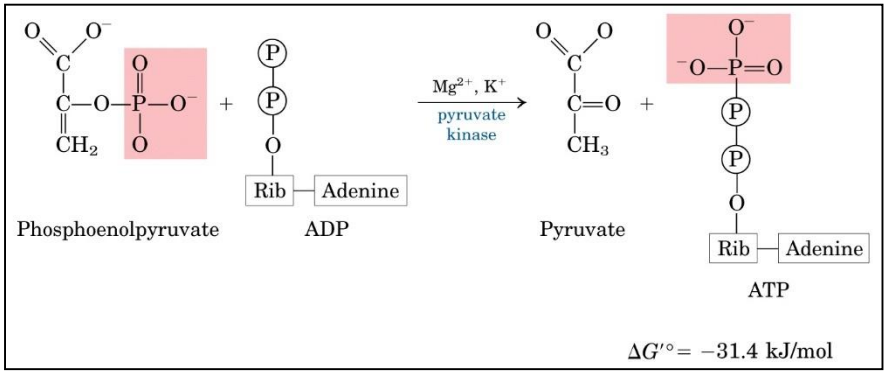


Phosphoproteinphosphatase 1

Phosphorylates pyruvate kinase

(Glycolysis is inhibits)

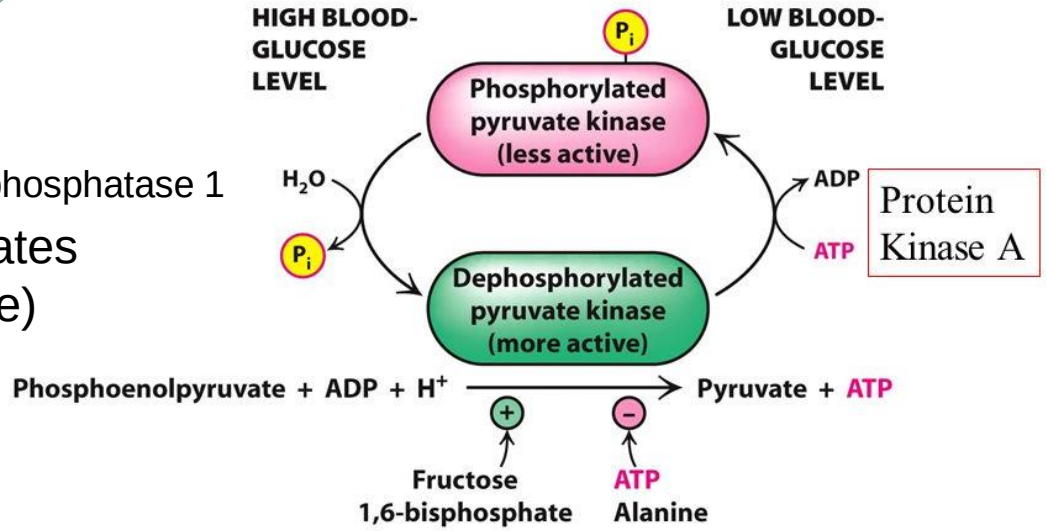
Pyruvate kinase regulation



- Hormonal regulation (insulin)

When [glucose] increases --→ insulin----→ phosphoproteinphosphatase 1

Phosphoproteinphosphatase 1 (dephosphorylates pyruvate kinase)

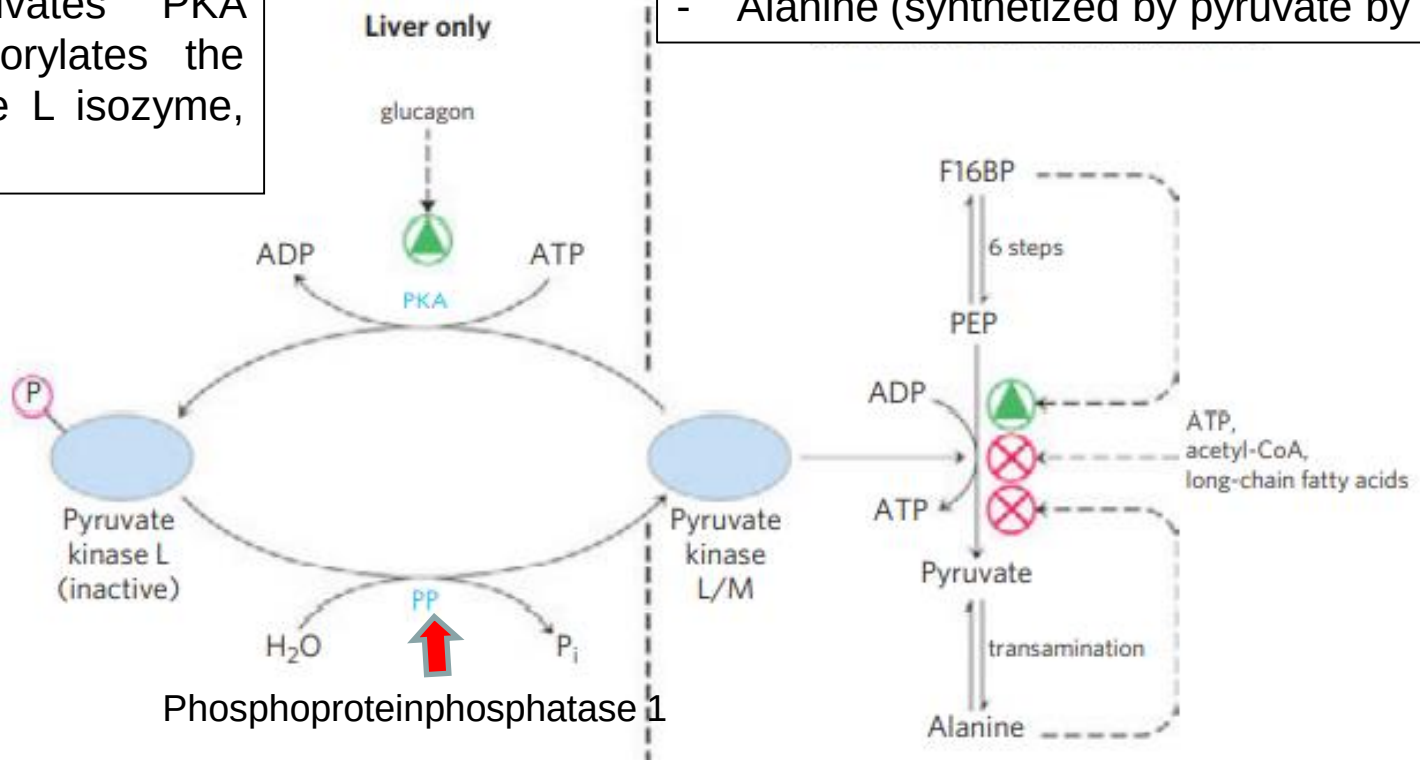


Glycolysis increases

Regulation of pyruvate kinase.

The liver isozyme (L form) is also regulated hormonally. Glucagon activates PKA (which phosphorylates the pyruvate kinase L isozyme, inactivating it.)

Allosteric inhibition of pyruvate kinase
- ATP
- Acetyl Co-A,
- Long chain fatty acids
- Alanine (synthetized by pyruvate by 1 step)



The muscle isozyme (M form) is not affected by this phosphorylation mechanism.

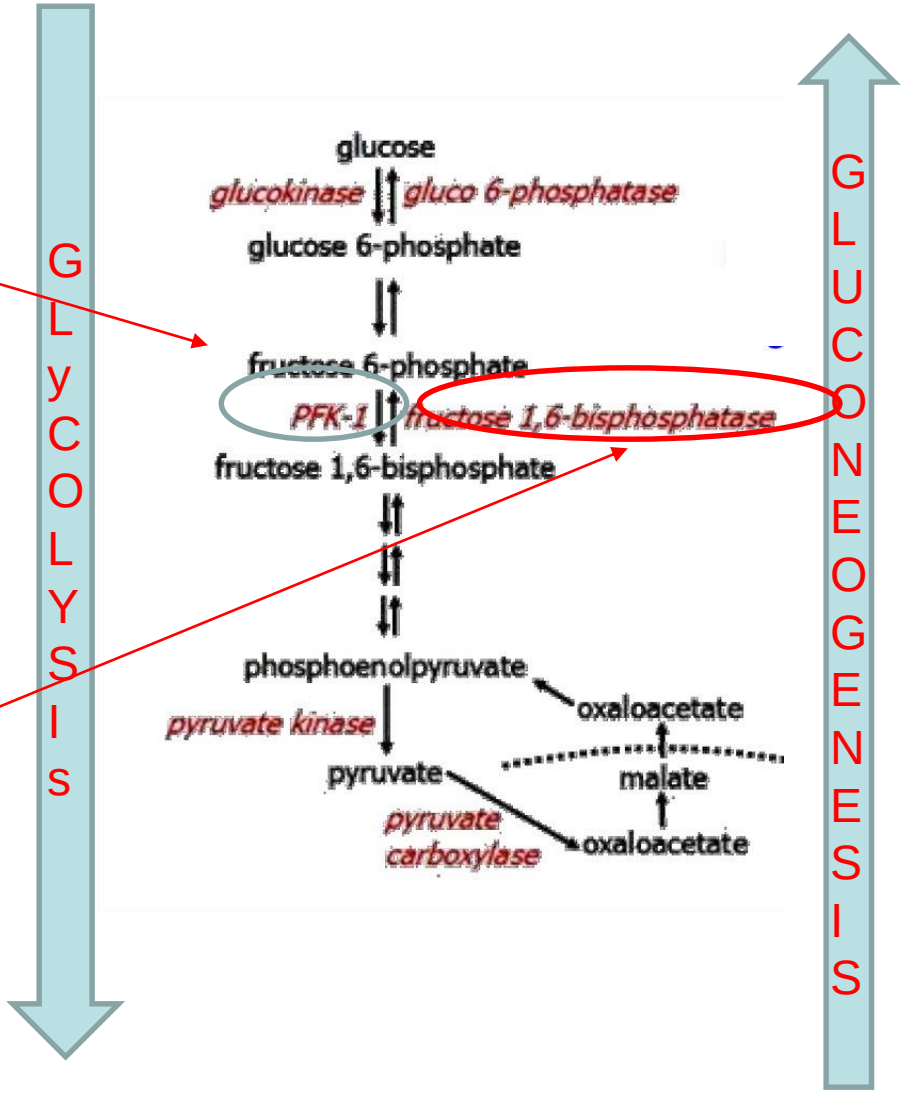
Accumulation of alanine, which can be synthesized from pyruvate in one step, allosterically inhibits pyruvate kinase, slowing the production of pyruvate by glycolysis.

Glycolysis

- Pyruvate kinase
- Phosphofructokinase 1 (PFK-1)
- Hexokinase

Gluconeogenesis

- **Pyruvate decarboxylase**
- **Fructose 1,6 biphosphatase**
- **Glucose 6-phosphatase**

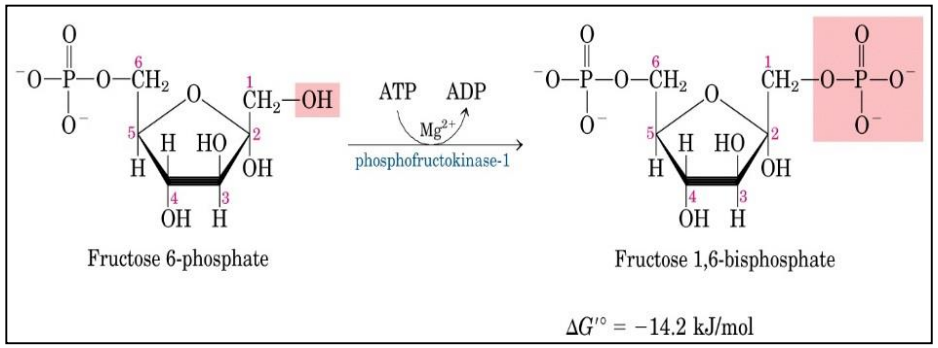


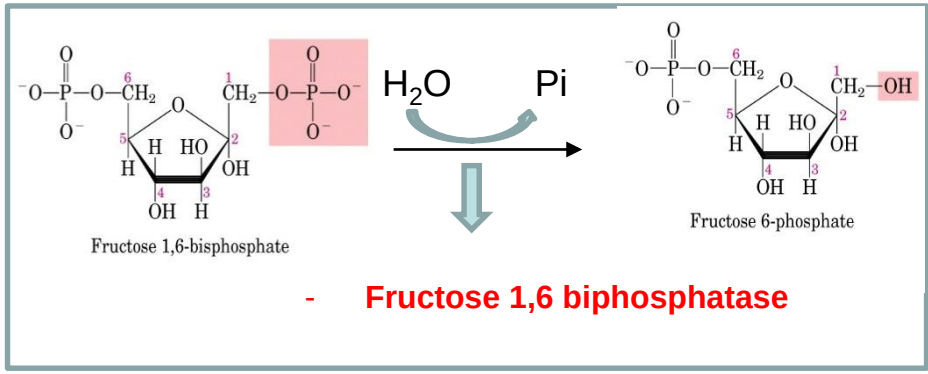
Second step to regulation

Phosphofructokinase-1 (PFK-1).

(glycolysis)

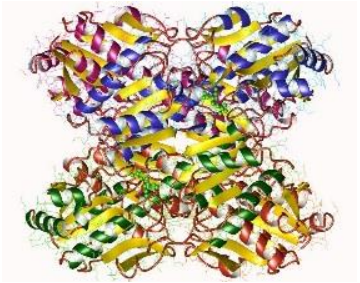
- **Fructose 1,6 biphosphatase (gluconeogenesis)**



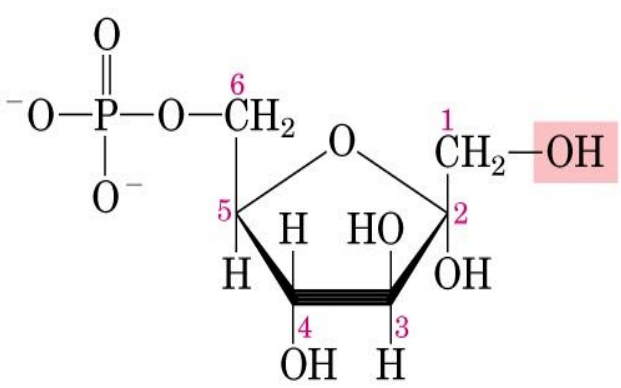


Phosphofructokinase-1 and Fructose 1,6-Bisphosphatase Are Reciprocally Regulated

Phosphofructokinase-1(PFK-1). (glycolysis)



The enzyme is a tetramer formed by two pairs of α and β subunits

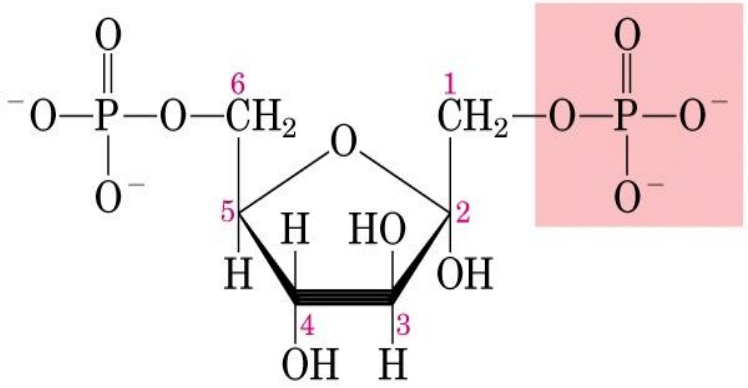


Fructose 6-phosphate

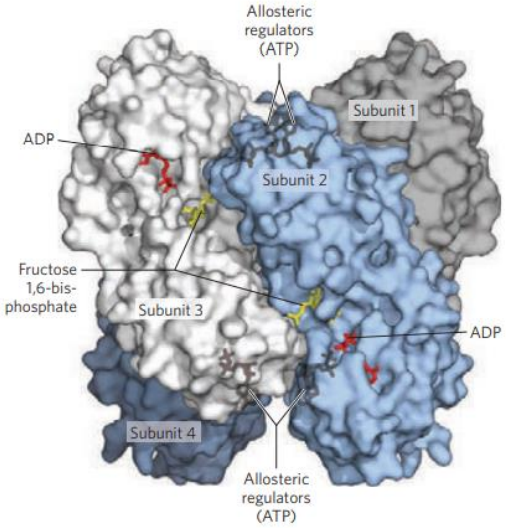
ATP ADP

Mg^{2+}

phosphofructokinase-1



Fructose 1,6-bisphosphate

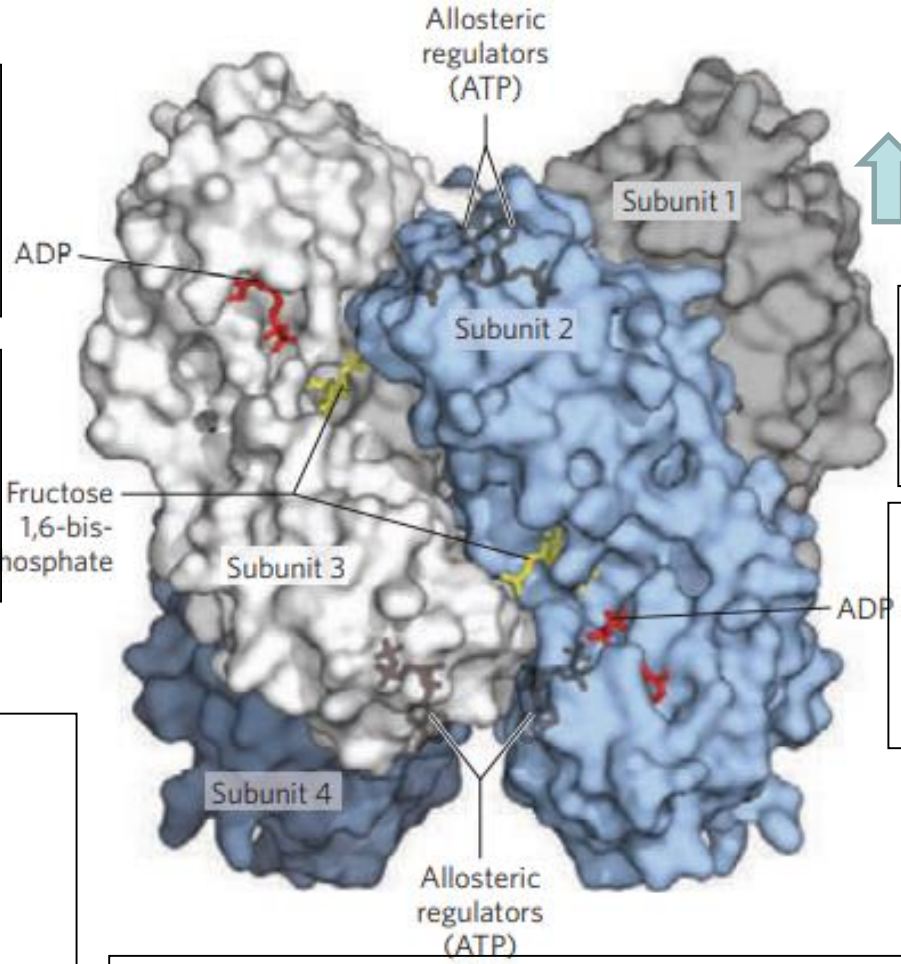


$\Delta G'^{\circ} = -14.2$ kJ/mol

Phosphofructokinase-1(PFK-1) (glycolysis).

Phosphofruttokinase-1 regolation has a complex allosteric reaction:

ATP is not only a substrate for PFK-1 but also an end product of the glycolytic pathway.



[ADP] or [AMP],

When the consumption of ATP exceeds the production

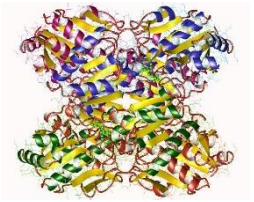
ADP and AMP act allosterically to relieve this inhibition by ATP..

cellular [ATP]

When ATP is produced faster than it is being consumed,

ATP inhibits PFK-1 by binding to an allosteric site and lowering the affinity of the enzyme for its substrate fructose 6-phosphate

Phosphofructokinase-1(PFK-1) (glycolysis).

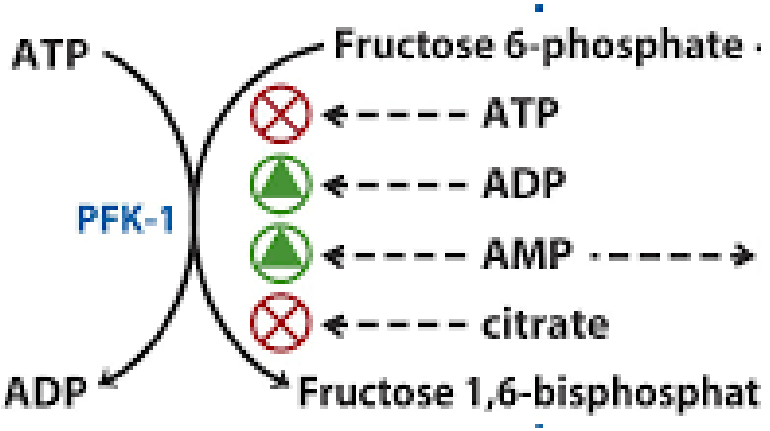


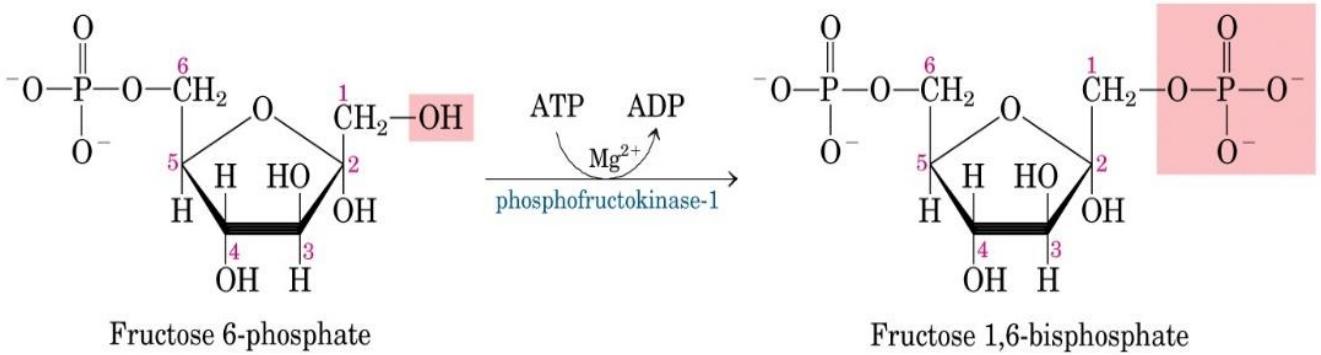
Phosphofruttokinase-1 regolation has a complex allosteric reaction:

-ATP, ADP e AMP

- Citrate
- Fructose 2,6 biphosphate

.





Fructose 6-phosphate

Fructose 1,6-bisphosphate

$\Delta G'^{\circ} = -14.2$ kJ/mol

Phosphofructokinase-1(PFK-1).

ATP allosteric regulation

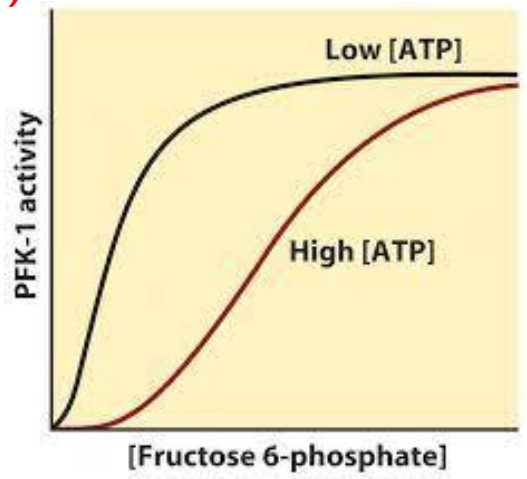
1. High ATP concentration…..Km increased (Km= 0,5)

ATP is negative modulator allosteric

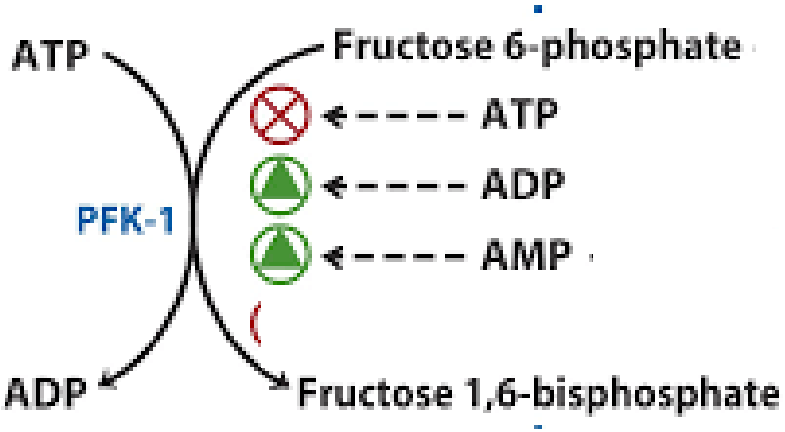
2. Low [ATP] = High [AMP] …..Km is minor to 0,5

- AMP is allosteric activator

Enzyme working to high velocity also in the presence of low substrate concentration.



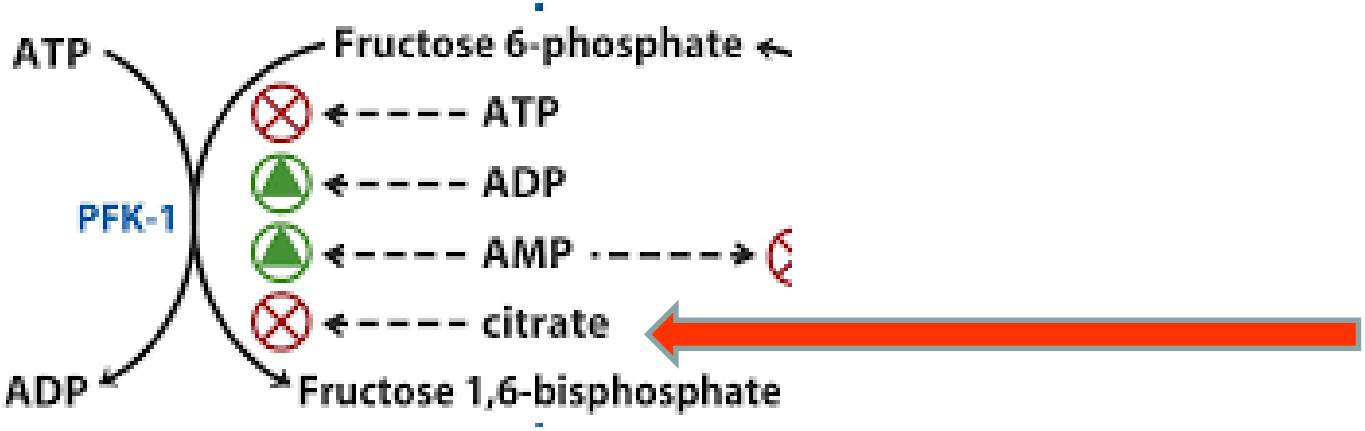
Cynetic of PFK-1 is sigmoidal:



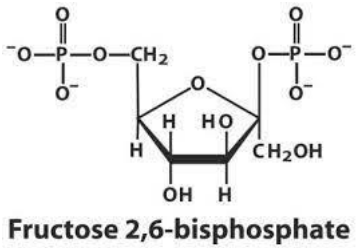
Citrate regulation of PFK-1 (negative modulator)

- Citrate, is key intermediate of aereobiotic oxidation of pyruvate, fatty acids and aminoacids

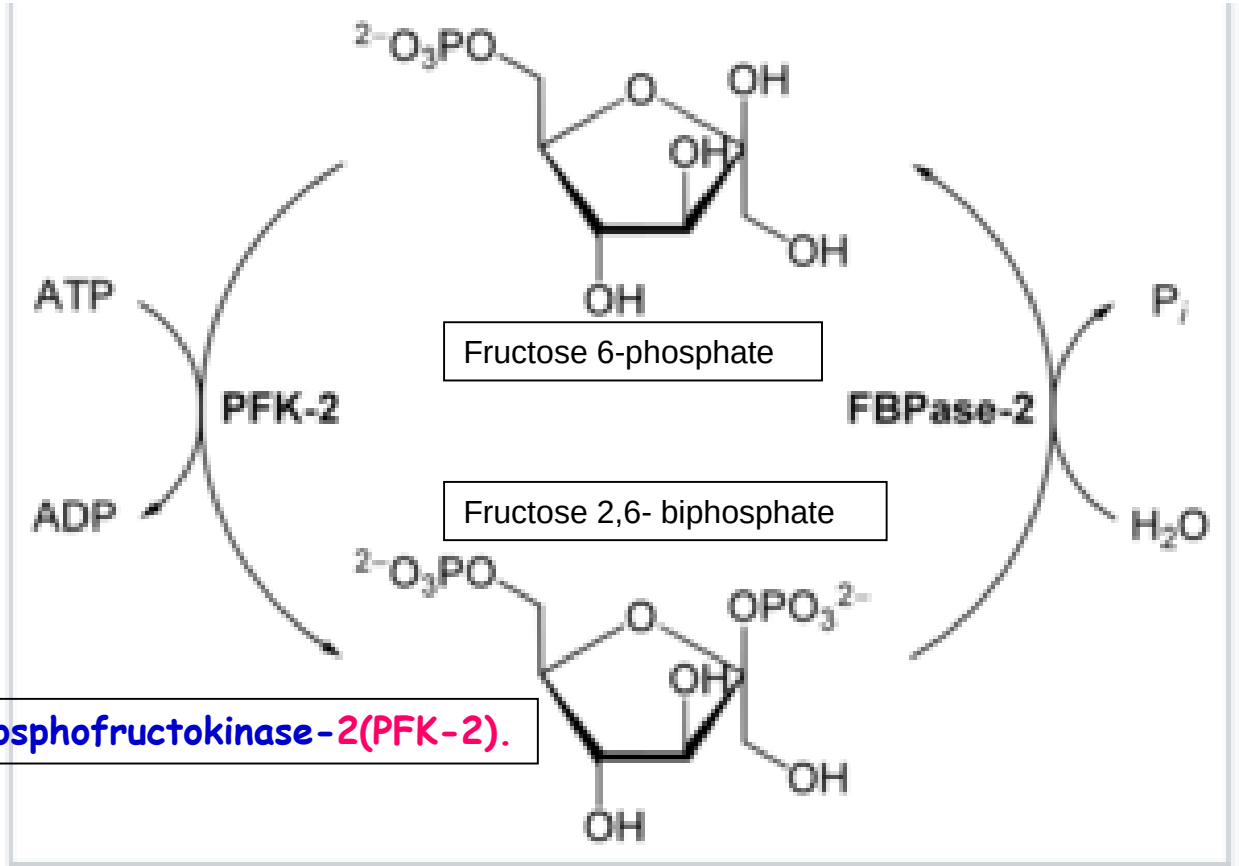
- High [citrate] indicate that energy storage is satisfied and decrease the glucose flow towards glycolysis



PFK-1 catalyzes the reaction only in the presence of fructose 2,6- biphosphate



Synthesis of fructose 2,6 biphosphate



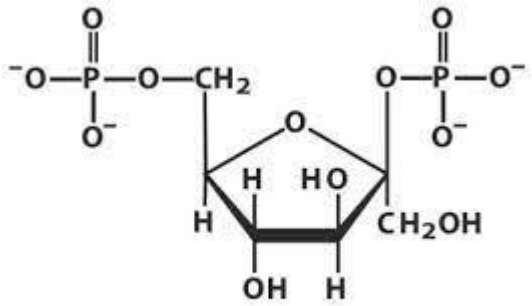
Riconverted to fructose 6-P by **fructose biphosphatase 2 (FBPase 2)**

Phosphofructokinase-2(PFK-2).

Fructose 2,6-biphosphate is allosteric activator of PFK-1)

Fructose 2,6-biphosphate is the principal modulator

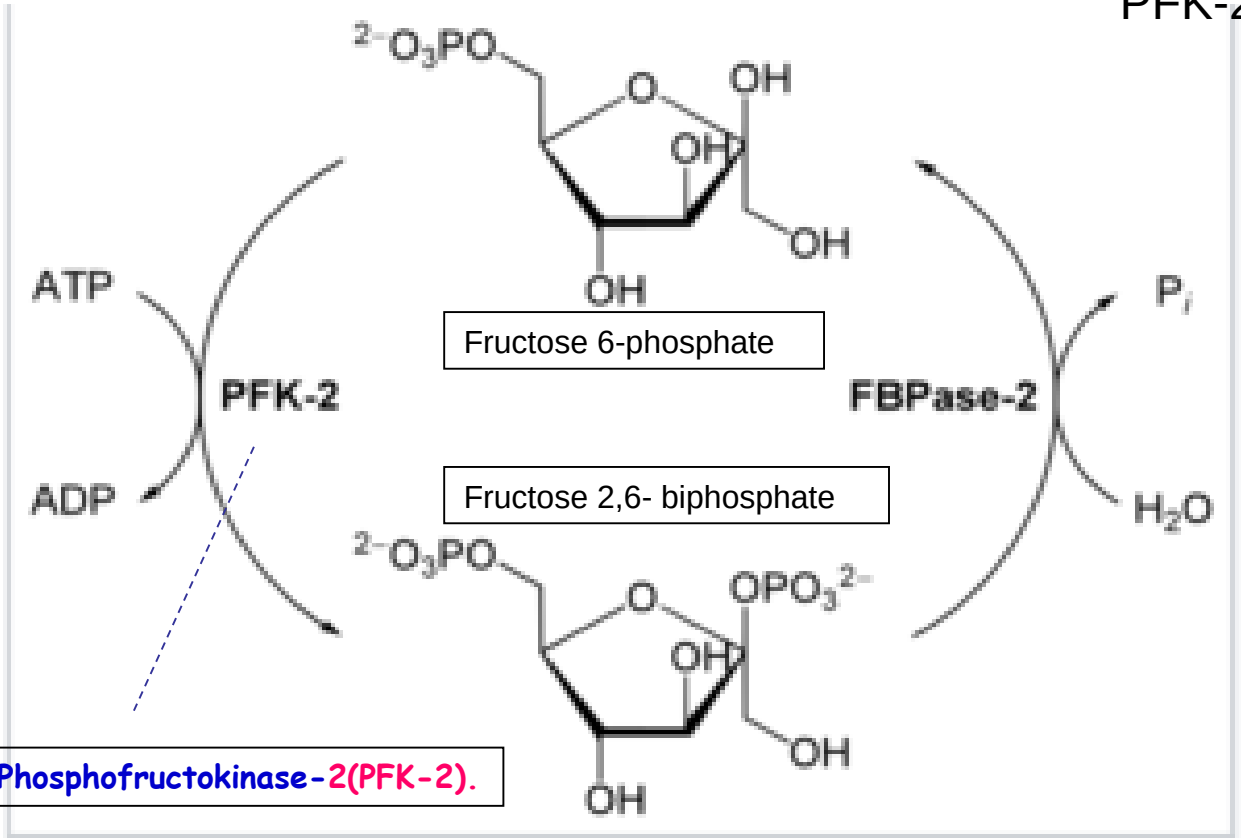
Key role in coordinated regulation between glycolysis and gluconeogenesis



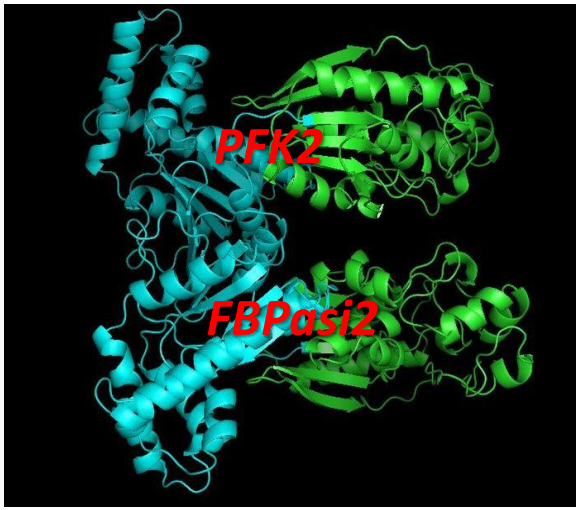
Fructose 2,6-bisphosphate

PFK-2 and FBPasi-2 are in the same polypeptide chain and form a tandem or bifunctional enzyme.

PFK-2 and FBPasi-2 are a dimer.



Phosphofructokinase-2(PFK-2).

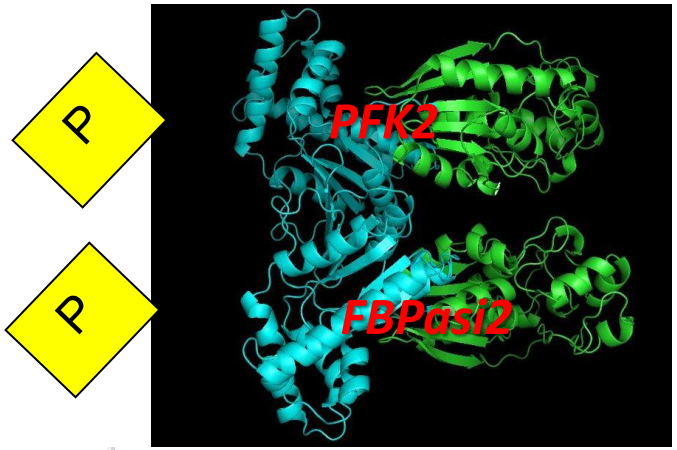


Activity is regulated by reversible phosphorilation

PFK2 and FBPasi 2 Activity are regulated by phosphorilation-dephosphorylation on N-terminal Ser_{32} residue in each monomer

Phosphorylated enzyme

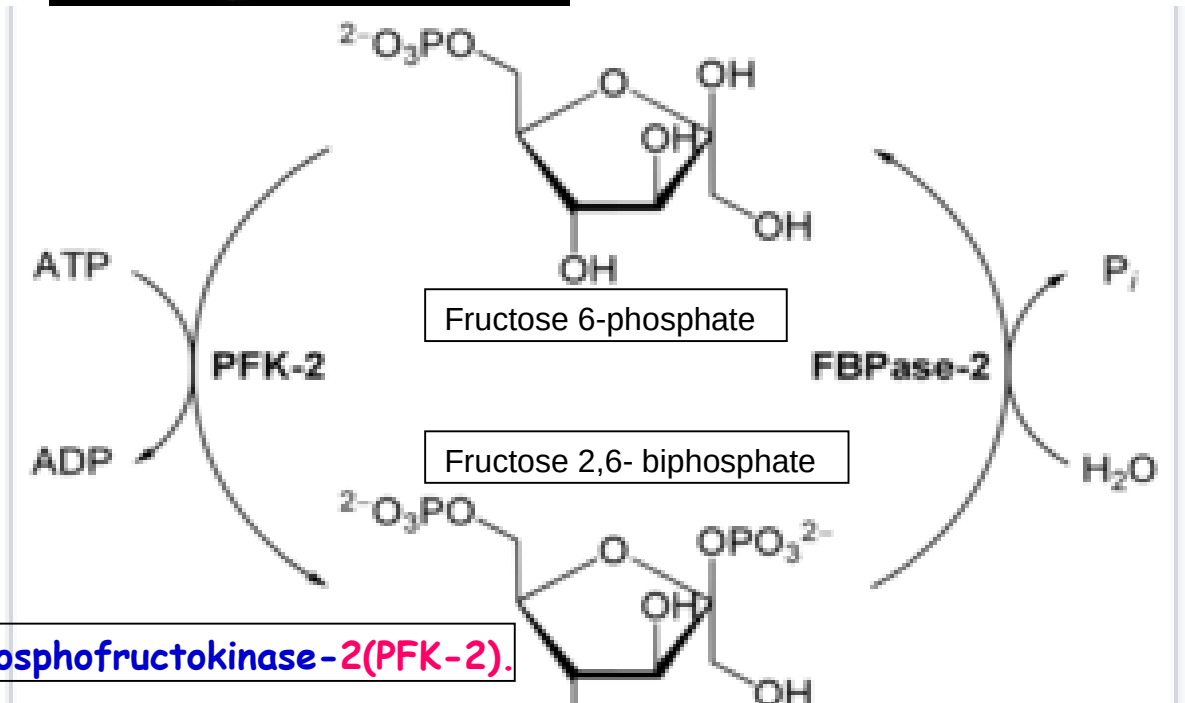
PFK2
Inactive



FBP-ase
Active

[Fructose 2,6 biphosphate]

Glycolysis is inhibited

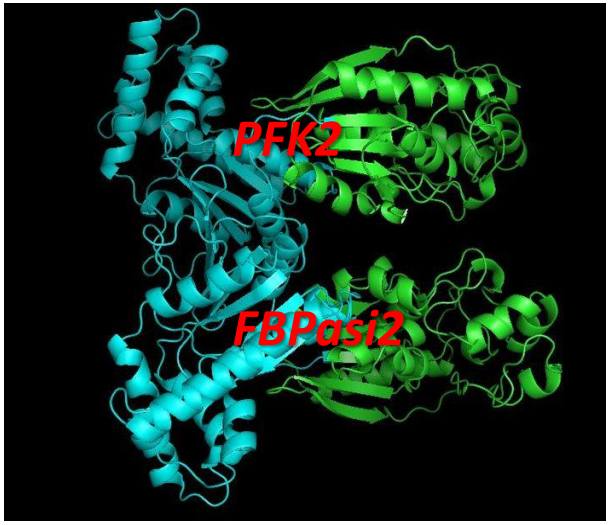


Phosphofructokinase-2(PFK-2).

PFK2 and FBPase 2 Activity are regulated by phosphorylation-dephosphorylation on N-terminal Ser_{32} residue in each monomer

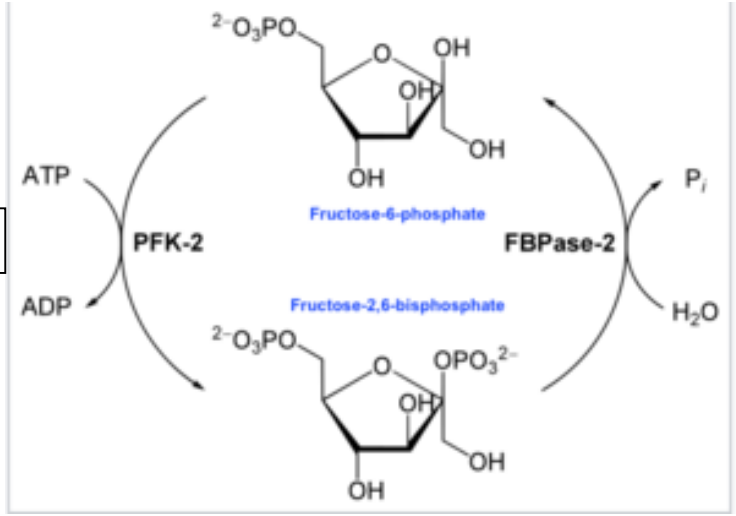
dephosphorylated enzyme

PFK2 Active



FBP-ase Inactive

[Fructose 2,6 biphosphate]



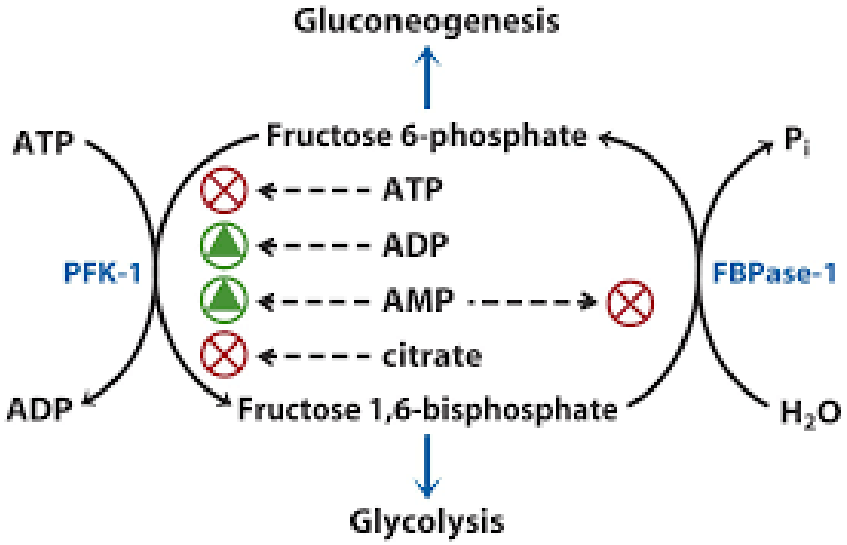
Flow is directed towards glycolysis

Fructose 2,6-biphosphate (allosteric activator of PFK-1)

Fructose 2,6-biphosphate

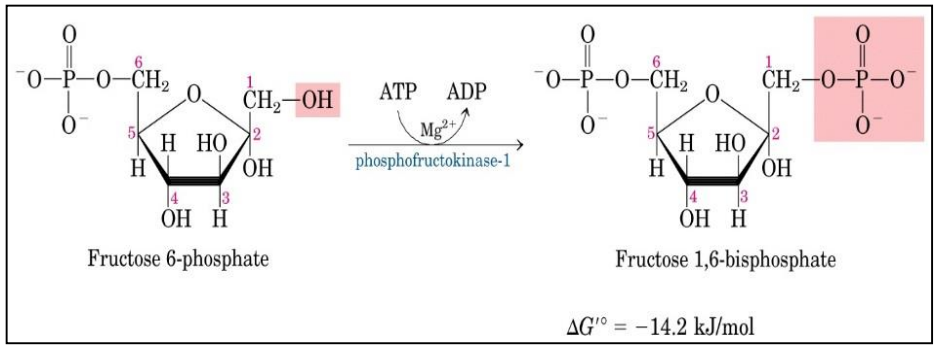
F2,6P

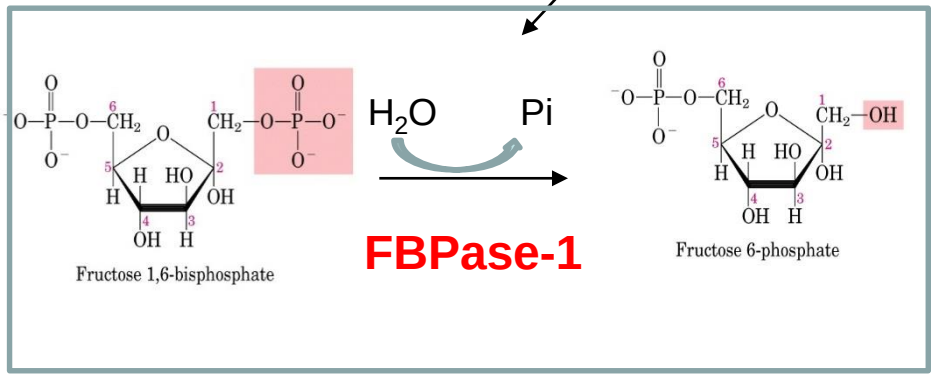
Activates PFK-1



F2,6P

Inhibits FBPase-1

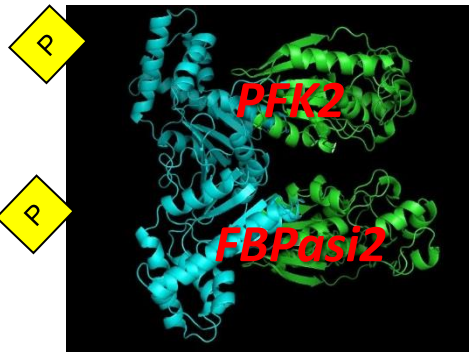




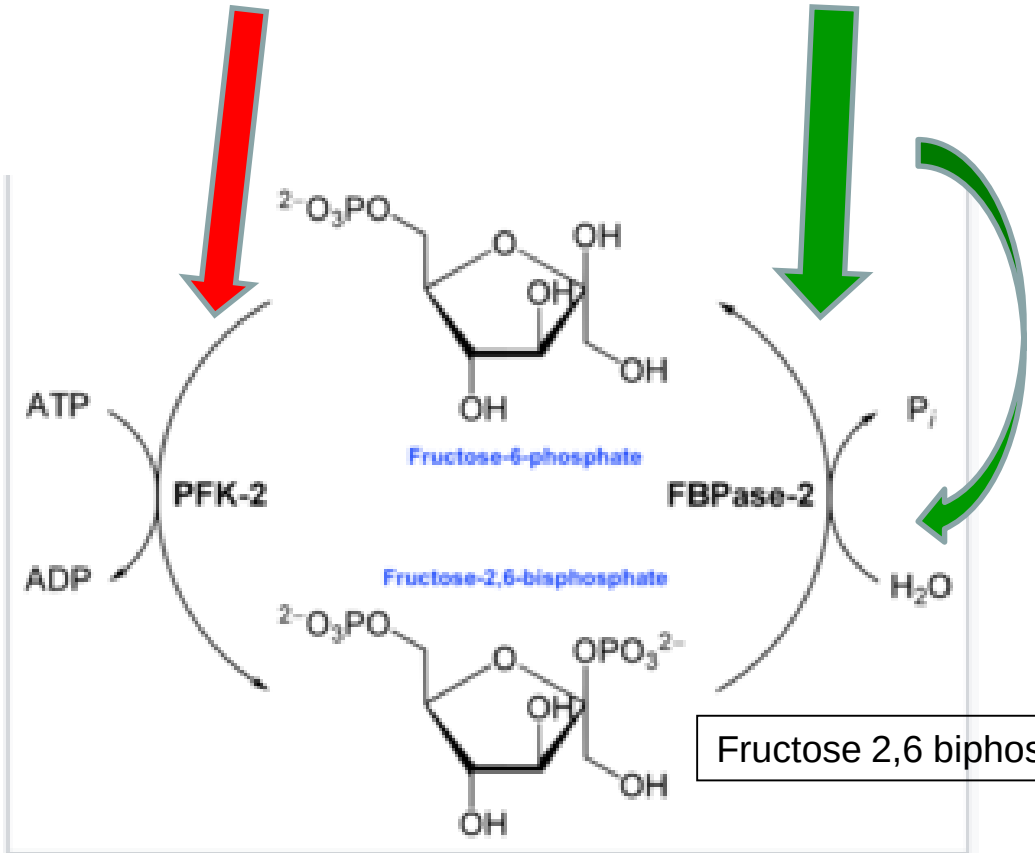
Hormonal Regolation

When [glucose] is low, glucagon stimulates the production of cAMP in hepatic cells
cAMP stimulates Protein kinase 2 (PK2) that phosphoryles bi-functional enzyme on Serine 32.

PFK2 inactive FBP-ase 2 active



PFK-2 is inactive and FBP-ase 2 active



Flow is directed towards gluconeogenesis

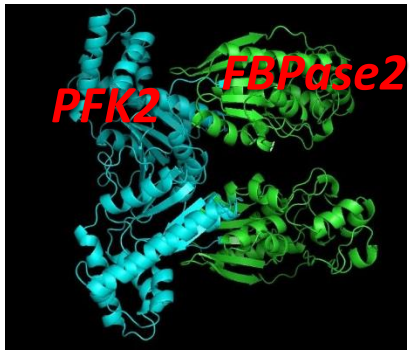
Fructose 2,6 biphosphate decrease

When [glucose] is high, decrease cAMP in hepatic cells.

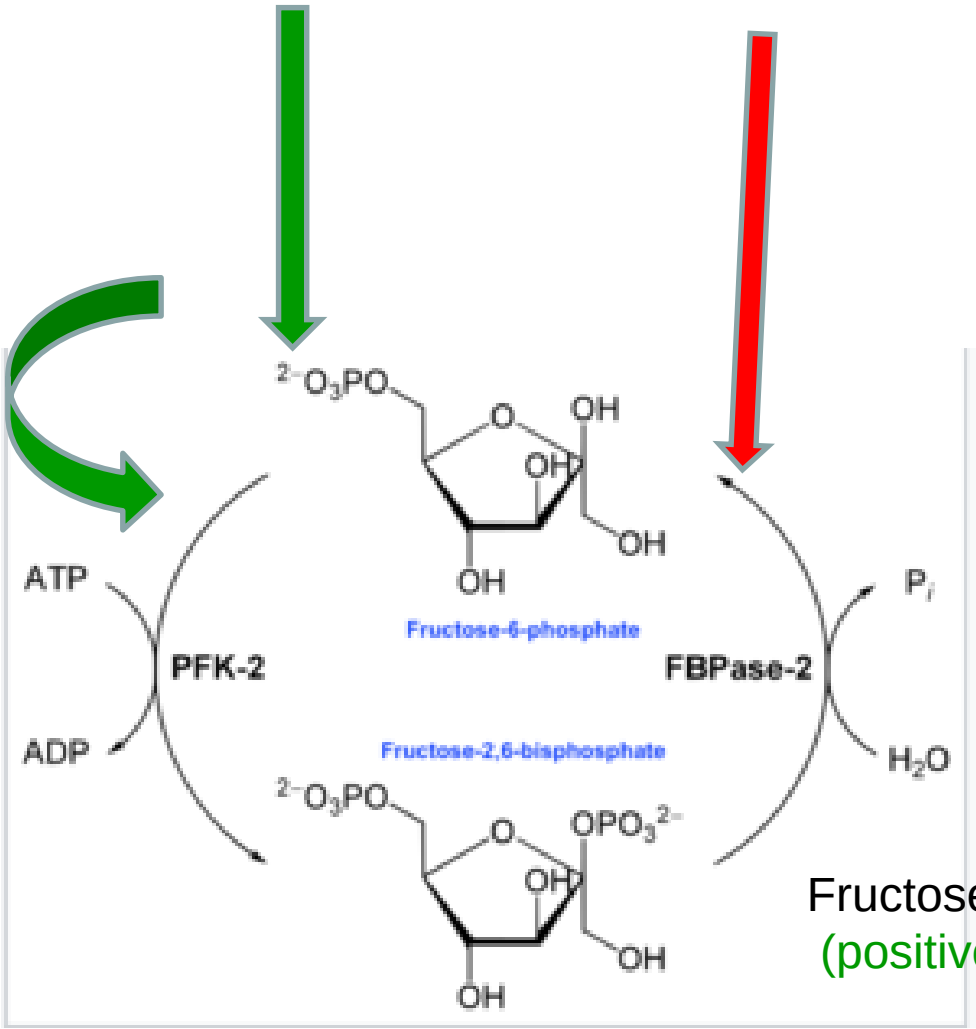
Bi-functional enzyme is dephosphorilated.

PFK-2 is active and FBP-ase2 is inactive

PFK2
Active

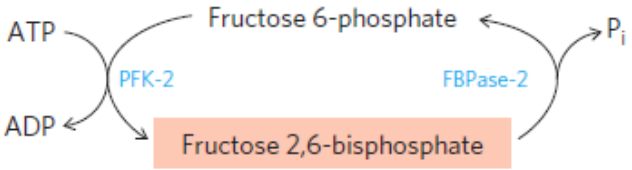


FBP-ase 2
Inactives

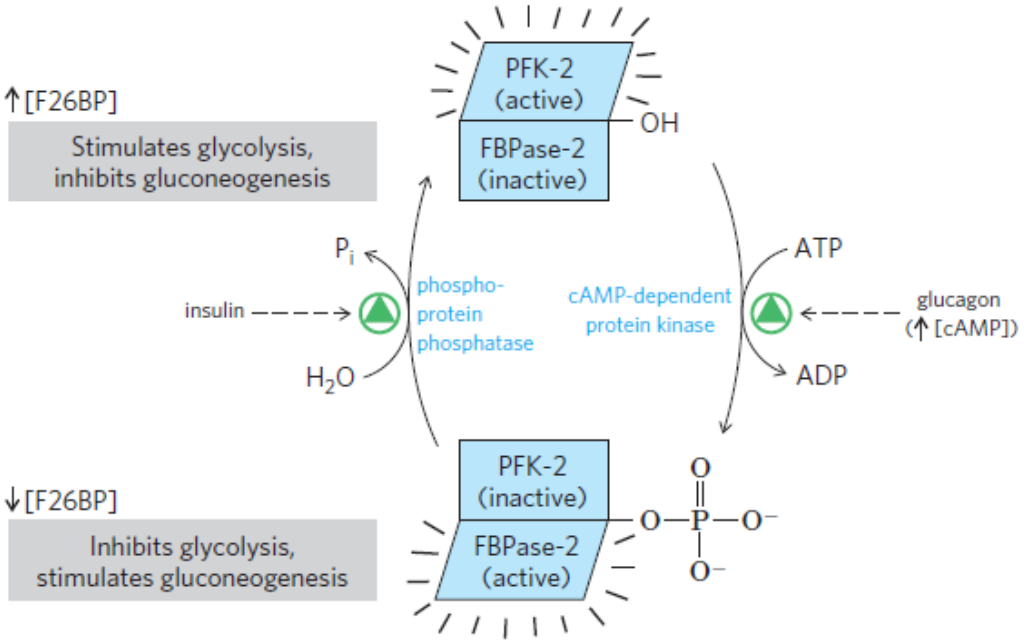


Flow is directed towards glycolysis

Fructose 2,6 biphosphate increases (positive modulator of PFK-1)


ATP
Fructose 6-phosphate
P_i
PFK-2
FBPase-2
ADP
Fructose 2,6-bisphosphate

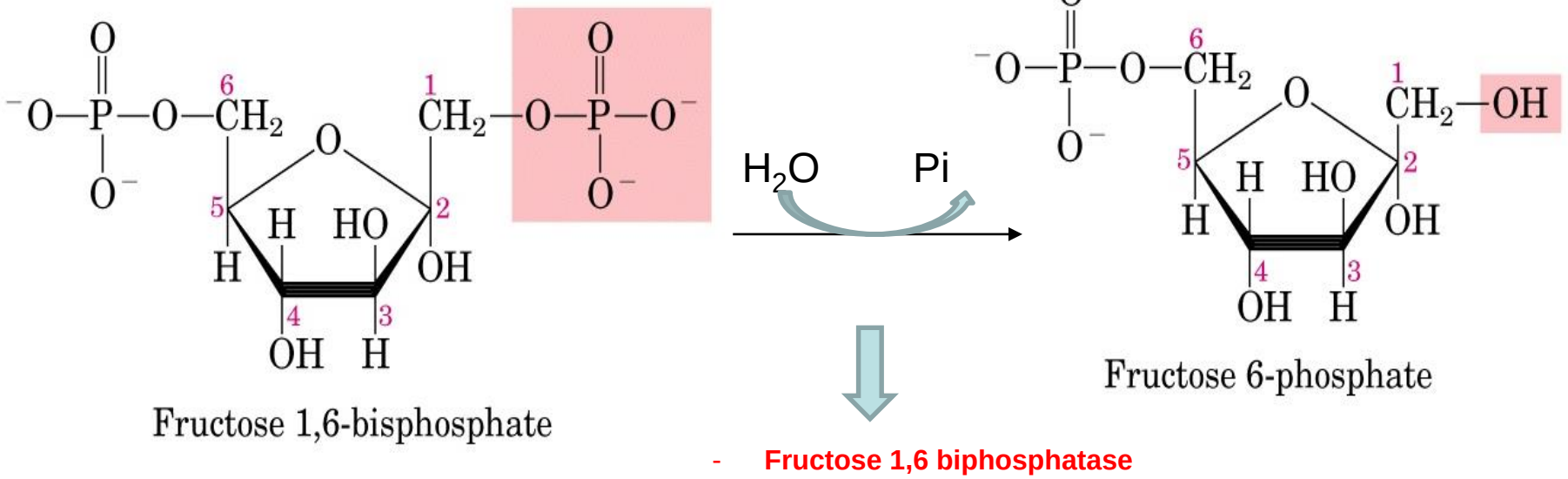
(a)


↑[F26BP]
Stimulates glycolysis, inhibits gluconeogenesis
PFK-2 (active)
FBPase-2 (inactive)
OH
P_i
insulin
H_2O
phospho-protein phosphatase
cAMP-dependent protein kinase
ATP
glucagon
(↑[cAMP])
ADP
↓[F26BP]
Inhibits glycolysis, stimulates gluconeogenesis
PFK-2 (inactive)
FBPase-2 (active)
O
O—P—O⁻
O⁻

(b)

- Fructose 1,6 biphosphatase

(gluconeogenesis)



- **Fructose 1,6 biphosphatase**

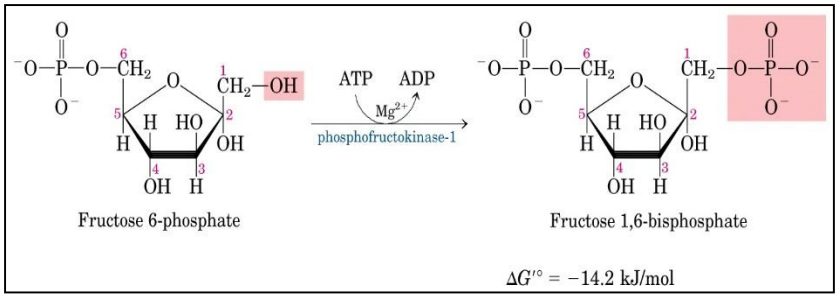
The enzyme shows sigmoidal kinetics and is allosterically inhibited by AMP and a regulatory molecule, fructose 2.6 bisphosphate

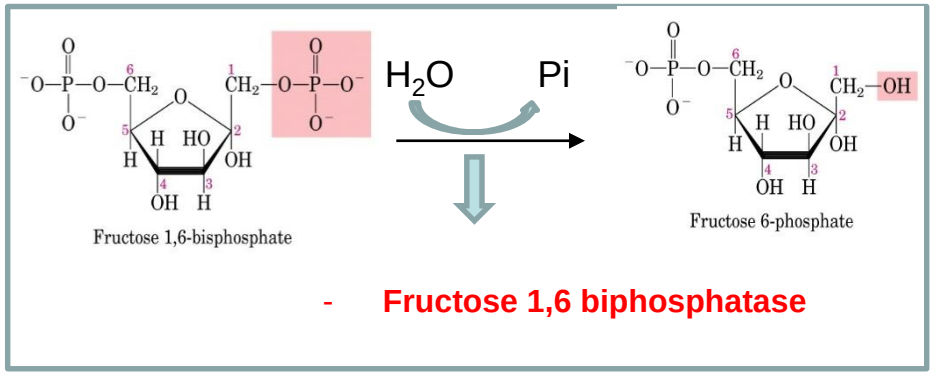
Second step to regulation

Phosphofructokinase-1 (PFK-1).

(glycolysis)

- **Fructose 1,6 biphosphatase 1 (gluconeogenesis)**





- **Fructose 1,6 biphosphatase**

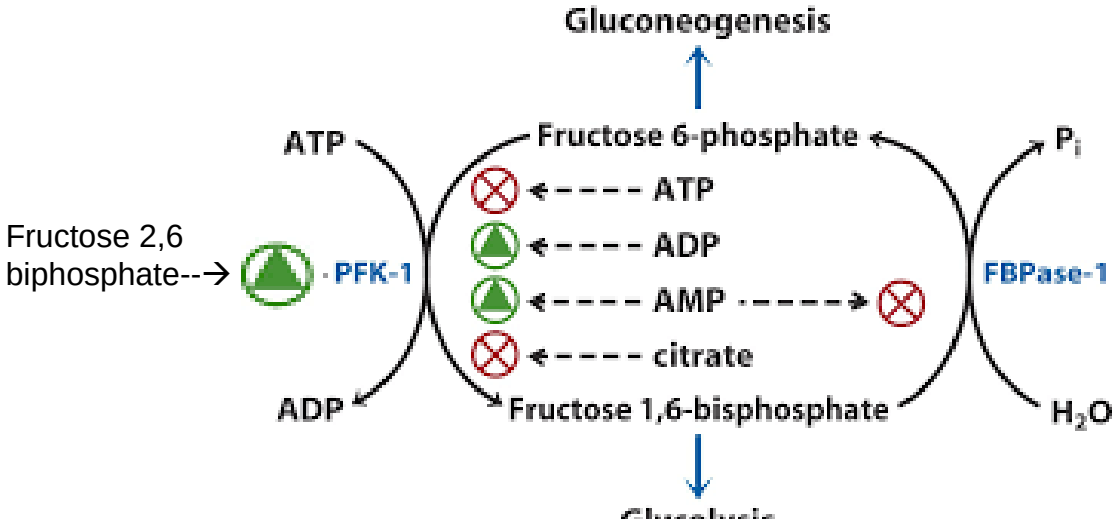
Inhibits from:

- **ATP**
- **Citrate**
- **Alanine**
- **Fatty acids**

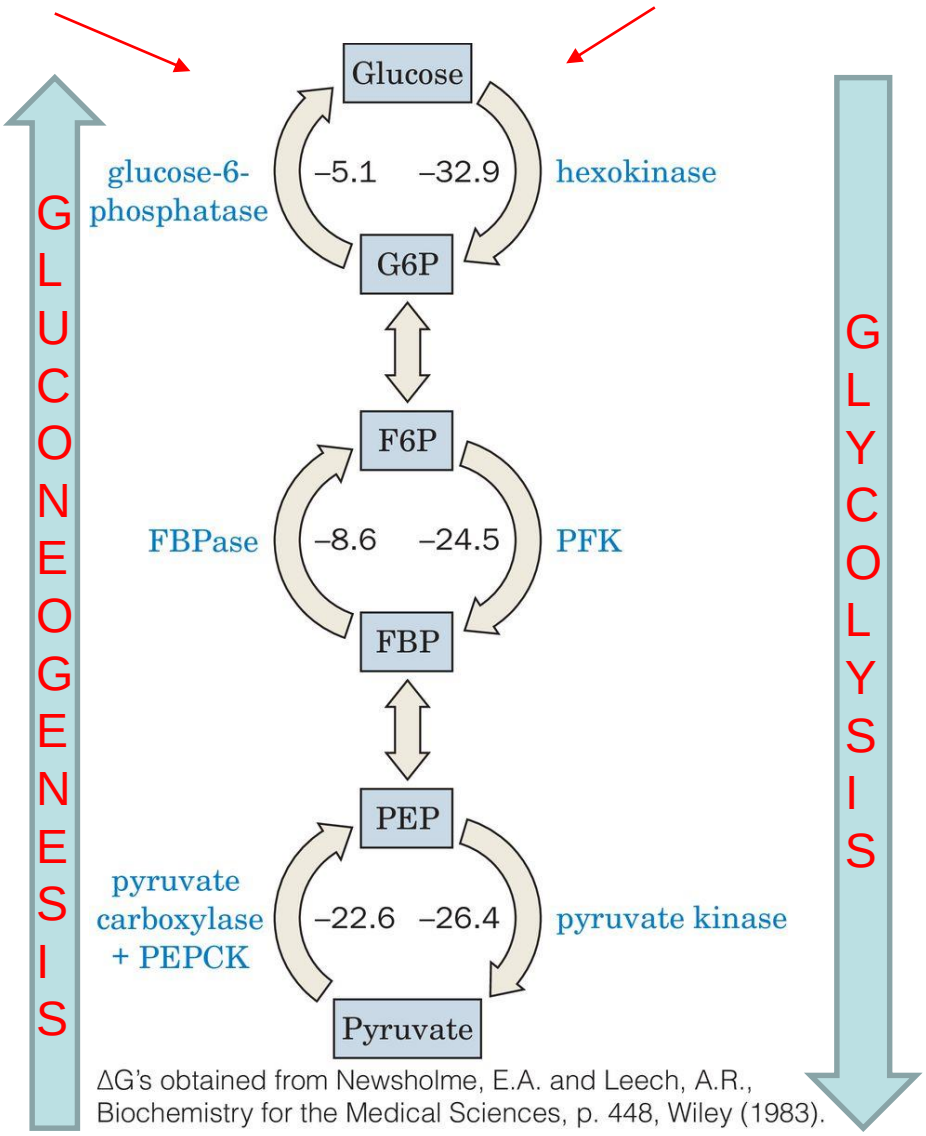
Inhibits from:

- **Fructose 2,6 biphosphate**
- **AMP**

- **Activated From :**
- **AMP, ADP**
- **Fructose 2,6 biphosphate**



Third step to regulation



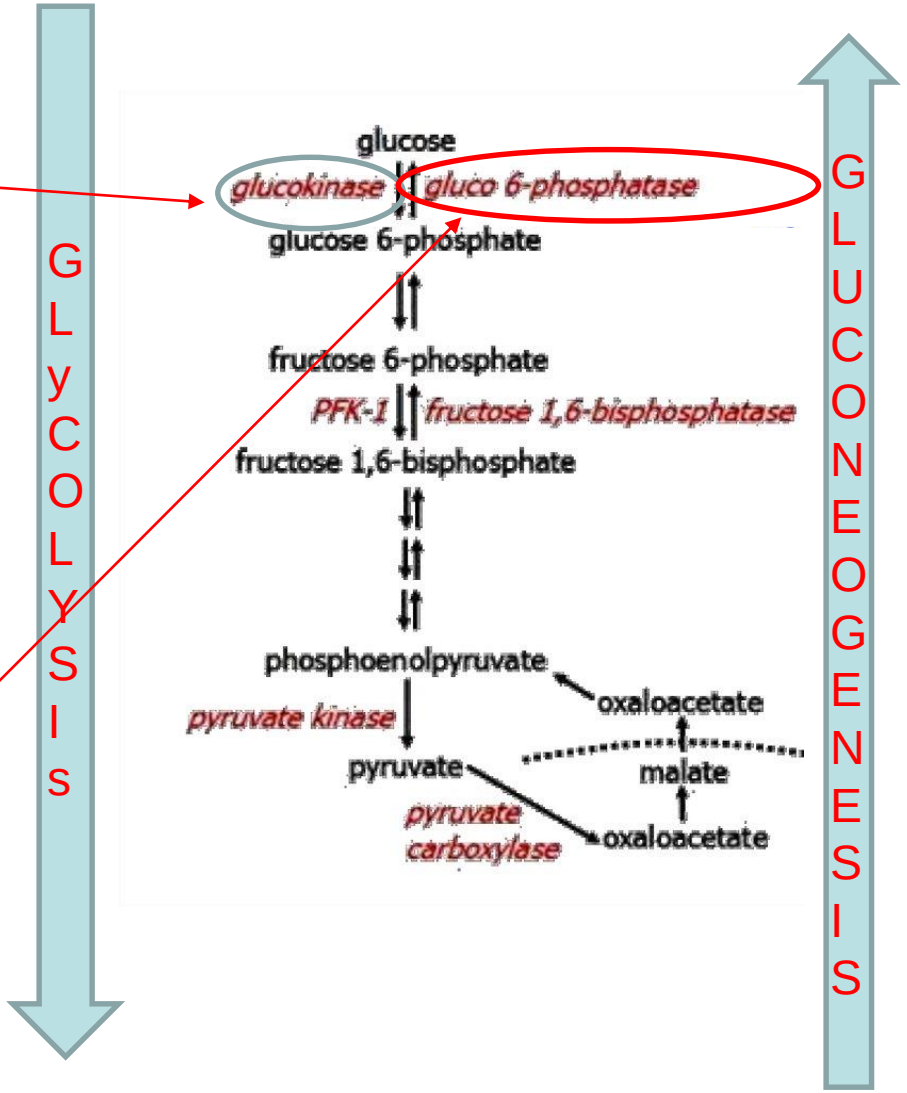
ΔG's obtained from Newsholme, E.A. and Leech, A.R., Biochemistry for the Medical Sciences, p. 448, Wiley (1983).

Glycolysis

- Pyruvate kinase
- Phosphofructokinase 1
- Hexokinase

Gluconeogenesis

- **Pyruvate decarboxylase**
- **Fructose 1,6 biphosphatase**
- **Glucose 6-phosphatase**

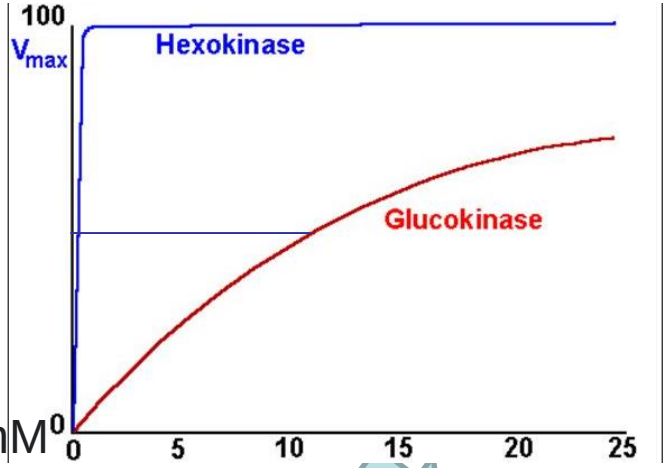


Hexokinase (glycolysis)

Glucose ---(Hexokinase)---> Glucose 6-P:

While in the muscle it is to produce energy

The role of glucose in the liver is to regolate blood sugar.
.



High affinity for glucose: since the glucose that enters the myocytes from the blood is sufficient to saturate the enzyme,
it always works at maximum speed

Km= 0,1mM
Muscle is low

Km=10 mM
Liver is high

Lower affinity for glucose.
the glucose concentration at which the enzyme is half saturated is higher than the normal concentration of glucose in the blood.

Few concentration of glucose are converted to glucose 6-poshate and sent towards glycolysis.

When concentration of glucose exceeds blood concentration (5mM), glucose sent towards glycolysis

Hexokinase (glycolysis)

Glucose ---(Hexokinase)---→ Glucose 6-P:

- Muscle and other tissue uses glucose to produce energy:
-

Hexokinases I, II and III are ihnibited from high Glucose 6-P concentrations

Muscle **hexokinases** are allosterically inhibited by their product,

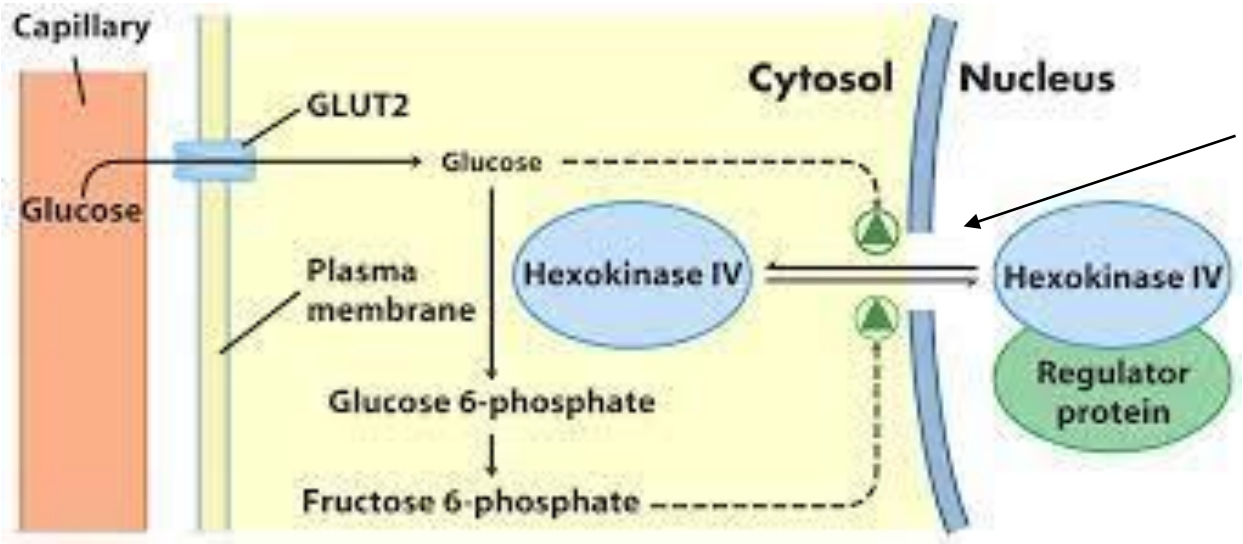
Liver maintains a constant blood glucose concentration:

Hexokinase IV (glucokinase) is not inhibited from glucose 6 P and phopsphorylates glucose also when the G6P concentration is high.

Glucokinase Regolation (liver)

When blood glucose is high excess glucose is transported (GLUT2 transporter) into hepatocytes, where hexokinase IV converts it to glucose 6-phosphate.

Glucose enters the hepatocyte by GLUT 2 transporter and is directed to the glycolytic pathway.



Nuclear pores

Hexokinase IV binds a regulator protein

Expressed in hepatocytes and localized in the nucleus

Glucose competes with fructose 6-phosphate for binding and causes dissociation of the regulatory protein from the hexokinase, Hexokinase migrates from nucleus to cytosol and phosphorylates glucose

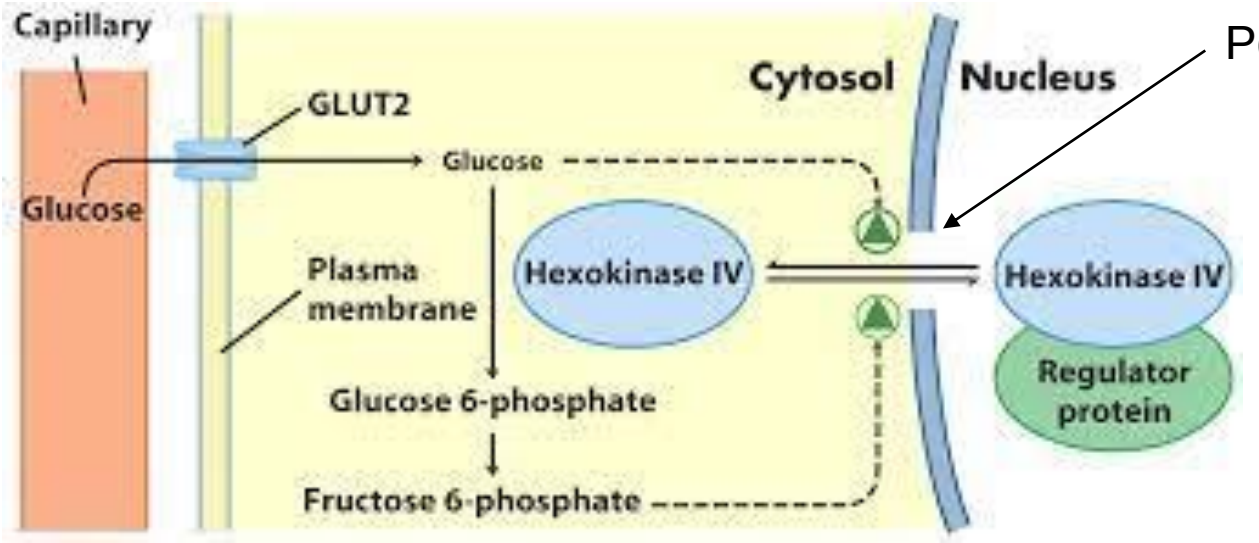
Glucokinase Regolation

Hexokinase IV is subject to inhibition by the reversible binding of a regulatory protein specific to liver

At low concentration of glucose

Fructose 6-phosphate triggers the inhibition of hexokinase IV by the regulatory protein,

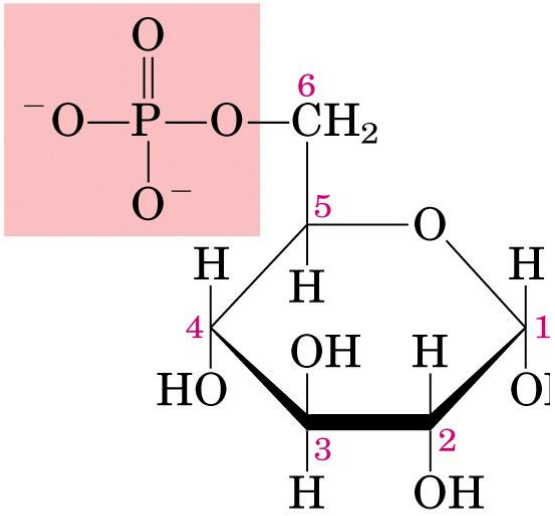
Hexokinase are sequestrated in the nucleus and glycolysis is inhibited

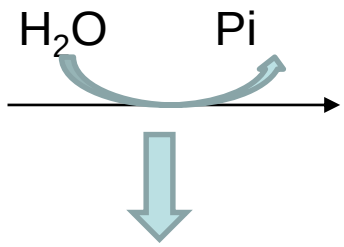


Pori nucleari

- **Glucose 6-phosphatase (gluconeogenesis)**

Found only in the liver, kidneys and small intestine

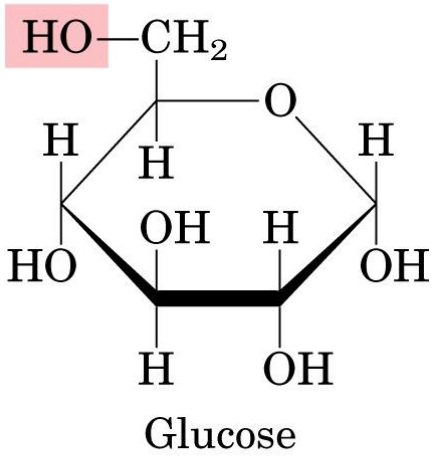




Glucose 6-phosphatase

it is linked to the endoplasmic reticulum with its active site exposed to the lumen.

The enzyme is part of a complex (glucose 6 phosphate transporter (G6PT) and a phosphate transporter.



Is carried to the cell surface and sent to the bloodstream

G6PT transports G6P from the cytosol into the RE where it is hydrolyzed to glucose and Pi.

- **Glucose 6-phosphatase has not allosteric regulation**
- **Regulated only substrate concentration**

is carried back by the transporter to the cytosol

Hexokinase IV (Glucokinase) and Glucose 6-Phosphatase are Transcriptionally Regulated

- Hexokinase IV is also regulated at the level of protein synthesis.

-greater energy production
(low [ATP],
high [AMP],
vigorous muscle contraction)

- greater glucose consumption
(high blood glucose)

increased transcription of the hexokinase IV gene.

- Glucose 6-phosphatase is transcriptionally regulated

necessary production of glucose

(low blood glucose, glucagon signaling).